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BIOLOGY AND IMPACT OF ZEBRA MUSSELS IN LAKE ST. CLAIR AND LAKE ERIE

RAC Project No. 443G
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LAKE ST. CLAIR AND LAKE ERIE

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FINAL REPORT

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SHELL FEATURES OF *DREISSENA POLYMORPHA* AND *MYTILOPSIS LEUCOPHAETA* (BIVALVIA: DREISSENIDAE) IN NORTH AMERICA

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SUMMARY

Dreissena polymorpha, recently introduced to freshwater habitats of North America, has been confused with *Mytylopsis leucophaeta*, a related species that is native to brackish and freshwaters of North America. Confusing features include similar attachment mechanisms, shape and colouring, including colour patterns between the two species. To help distinguish the two species, internal and external features, ultrastructure and composition of *D. polymorpha* and *M. leucophaeta* shells were determined using stereoscope, scanning electron microscopy and X-ray diffraction techniques. Samples of *D. polymorpha* from Lake St. Clair and Lake Erie, Ontario, and samples of *M. leucophaeta* from the Patapsco River, a tributary of Chesapeake Bay, Maryland, were examined. The most reliable diagnostic feature is the presence of a small triangular shaped tooth, an apophysis, that extends from the myophore plate, in *M. leucophaeta* and its absence in *D. polymorpha*. Even though *D. polymorpha* and *M. leucophaeta* do occasionally have similar shell shapes and colour patterns, some external features can reliably separate the two species. *Dreissena polymorpha* shells have an acutely pointed umbone, are acutely angled ventro-posteriorly, and have a flattened, arched ventral surface with a prominent ventro-lateral shoulder or ridge. *Mytylopsis leucophaeta* shells have a rounded umbone, are rounded ventro-posteriorly and lack both a flattened, arched ventral surface and a prominent ventro-lateral shoulder or ridge. Both *D. polymorpha* and *M. leucophaeta* have an outer crossed-lamellar shell structure with an inner complex crossed-lamellar shell structure with a thin, prismatic pallial myostracum between. Microtubules, minute cylindrical perforations of a few microns in diameter, are more prominent in *M. leucophaeta* than in *D. polymorpha*. Shells of both *D. polymorpha* and *M. leucophaeta* are composed entirely of aragonite crystals.

INTRODUCTION

The study of the molluscan shell has contributed perhaps the largest fraction of literature on the Mollusca (Wilbur 1964), yet little is known about the comparative shell morphology between Dreissena polymorpha and Mytylopsis leucophaeta, the only two species of freshwater bivalves of the family Dreissenidae found in North America.

Dreissena polymorpha, the zebra mussel, is an exotic bivalve species that was introduced into the Great Lakes five years ago from the ballast water of freighters originating in Europe (Hebert et al 1989). Zebra mussels are now found in all the Great Lakes, and the St. Lawrence River and have begun to invade inland water systems in Canada and the United States. Zebra mussels use byssal threads to attach to almost any hard substrate, have already caused severe economic problems for "users" of Great Lakes water and pose a threat to the survival of natural inhabitants of the Great Lakes (Lewandowski 1976, Mackie et al 1989 and Mackie 1991). The earliest studies show that most adults live for only two years and have very rapid growth rates in their first year, and attain maximum shell lengths averaging 2 - 2.5 cm (Mackie 1991).

Mytylopsis leucophaeta, Conrad's false mussel (Mondadori 1980) or the platform mussel (Morris 1975), is a brackish to freshwater mussel native to North America that is found on the East coast from New York to Florida, and in Texas and Mexico (Mondadori 1980). Mytylopsis leucophaeta colonized the Hudson River, New York, in the 1930's as a result of ship ballast water discharge and has recently been discovered in the Upper Mississippi River (Koch 1989).

Mytylopsis leucophaeta ranges in size from 1 - 2 cm (Abbot 1974, Emerson and Jacobson 1976, Pennak 1978) and like D. polymorpha uses byssal threads to attach to hard substrates. Mytylopsis leucophaeta poses a similar threat to that of the zebra mussel, however not as severe (Mackie et al 1989).

The free-swimming veliger larvae of the zebra mussel enables it to spread rapidly downstream at a rate of up to 250 km per year (Mackie 1991). The zebra mussel's attachment to boat hulls ensures its eventual spread upstream and inland. This rapid means of dispersal may result in the "invasion" of D. polymorpha into the known habitat range of M. leucophaeta because the salinity tolerances for these two species appear to overlap greatly between 0.2 and 3.0 ppt total salinity (MacNeill 1991a, 1991b). There is a known habitat overlap of D. polymorpha and M. leucophaeta in the estuarian delta of the Rhine, Meuse and Scheldt Rivers in Northwestern Europe (Wolff 1969).

There has been some confusion in distinguishing M. leucophaeta from D. polymorpha because of similar shape and colouring, including colour patterns (Fig. 1a and 1b). While M leucophaeta is usually uniformly dark with no stripes (Fig. 1c), some specimens have distinct stripes (Fig. 1b) much like

D. polymorpha (Fig. 1a). In addition, not all zebra mussels are striped in appearance. Some are all black, brown or white (Fig. 2). With the potential range overlap for these two species, in areas such as the Hudson and Mississippi Rivers, it is important to determine reliable diagnostic shell features in order to separate the two species.

The objectives of this study were to determine the differences between D. polymorpha and M. leucophaeta in: (1) internal and external diagnostic shell features; (2) shell ultrastructure; and (3) shell composition using stereoscope, scanning electron microscopy, and X-ray diffraction techniques.

MATERIALS AND METHODS

Samples of D. polymorpha were collected from Lake St. Clair at Puce, Ontario and from Lake Erie at Port Stanley, Ontario; samples of M. leucophaeta were collected from the Patapsco River of Maryland, a tributary of Chesapeake Bay. Laboratory observations were made with a Wild stereoscope at 5 - 32 x magnification.

The shell structure was determined by breaking both valves transversely by hand, then mounting all fragments on scanning electron microscope (SEM) stubs with the broken edges face up. Fragments were coated with gold paladium using a Hummer 2 sputter coater. The broken edge was examined and photographed with a JEOL 35C SEM from 250 - 2000 x. Ten shells from each site were examined.

Six to eight shells from each site were powdered and the powder press samples were analyzed using a Rigaku Geigerflex X-ray diffractometer to determine shell composition.

RESULTS AND DISCUSSION

Internal Diagnostic Shell Features

The most reliable diagnostic shell feature is internal. Mytylopsis leucophaeta possesses a small triangular shaped tooth, an apophysis, that extends from the myophore plate (Fig. 3a). In D. polymorpha no apophysis is present (Fig. 3b). The myophore plate, or septum, is located at the anterior end of the valve and is a place of muscle attachment (Figs. 3a and 3b). In M. leucophaeta the anterior adductor muscle attaches to the myophore plate while the anterior byssal retractor muscle attaches to the apophysis (Nuttall 1990) (Fig. 3c). In D. polymorpha both the anterior adductor and anterior byssal retractor muscles attache to the myophore plate (Fig. 3d). The anterior pedal-retractor muscle is a narrow band-like muscle that joins the dorsal part of the myophore plate in an elongate muscle scar

(Fig. 3d).

Morton (1970) and Nuttall (1990) do not clearly differentiate between pedal retractor and byssal retractor muscle scars on either the anterior or posterior end of the shell. In the D. polymorpha specimens that we examined there is no clear distinction between pedal retractor muscle and byssal retractor muscle scars either anteriorly or posteriorly. The same confusion exists with M. leucophaeta, as there appears to be no clear distinction between pedal retractor and byssal retractor muscle scars.

In D. polymorpha the posterior pedal-byssal retractor muscle is a massive muscle lying immediately dorsal to the foot and appears to retract both the foot and the byssus. Posterior to the pedal-byssal retractor muscle scar is the posterior adductor muscle scar (Fig. 3d).

Variations in the pallial line, a linear scar on the inner shell surface that marks the attachment of the mantle to the shell, also occur between these two species. In M. leucophaeta a slight indentation of the pallial line may be detected posteriorly and forms the pallial sinus (Fig. 3c). The pallial sinus marks the point of attachment of the siphon retractor muscle (Nuttall 1990). In D. polymorpha the pallial line (Fig. 3d) is normally described as being entire (Nuttall 1990) or more rounded and well defined with no pallial sinus present (Morton 1970).

External Diagnostic Shell Features

Even though D. polymorpha and M. leucophaeta occasionally have similar shell shapes and colour patterns (Fig. 1a and 1b), there are some external features that can separate the two species. Dreissena polymorpha shells have an acutely pointed umbone and are acutely angled ventro-posteriorly. The ventral surface is arched and flattened, usually with an acute shoulder or ridge ventro-laterally (Fig. 1a). Mytylopsis leucophaeta shells have a rounded umbone and are rounded ventro-posteriorly. The ventral surface is not arched and lacks a ventro-lateral shoulder or ridge (Fig. 1b).

Shell Ultrastructure

The bivalve shell is a complex organic/inorganic system consisting of two valves joined by a largely organic ligament. Each valve consists of an outer periostracum composed of organic material, conchiolin, and an inner calcified shell composed of calcium carbonate in an organic matrix (Kennedy et al 1969).

Both D. polymorpha and M. leucophaeta exhibit the same type of shell ultrastructure. There is an outer crossed-lamellar structure and an inner complex crossed-lamellar structure with a thin prismatic pallial myostracum between (Figs. 4 and 5). This corresponds to descriptions in European literature by Kennedy et al (1969), Taylor et al (1973), Denis (1979), and Archambault-Guezou (1982). The exact ages of the shells examined were not known. It is possible that the outer crossed-lamellar layer, between the periostracum and the pallial myostracum, is the shell growth for the first year, and that the inner complex crossed-lamellar layer, between the pallial myostracum and the inner shell surface, is the shell growth for the second year (Figs. 4 and 5).

Microtubules, minute cylindrical perforations of a few microns in diameter, were present in 90% of M. leucophaeta shells (Fig. 5) and present in only 10% of D. polymorpha shells examined. None of the M. leucophaeta specimens we examined had microtubules penetrate the periostracum. This corresponds with Siddall's (1980) observations that M. leucophaeta has evenly-dispersed and uniformly shaped microtubules that penetrate the inner calcareous crossed-lamellar layer but do not penetrate to the periostracum of the shell (Fig. 5). This is in disagreement with Kennedy et al (1969) who state that the microtubules found in tubulate bivalve shells do penetrate the periostracum. According to Siddall (1980), the microtubules are restricted to the older regions of the shell bounded by the pallial "line" or myostracum. This study found the microtubules to be restricted to the area between the periostracum and the pallial myostracum, the region that may represent the first year of growth and the oldest part of the shell.

The inorganic calcium carbonate crystals and organic matrix aggregate together into seven distinct but regularly occurring structures (Taylor et al 1969). The shell may be made up of several layers each formed from one of these structures. Nacreous, prismatic, foliated, crossed-lamellar, complex crossed-lamellar, and homogenous structures represent six of the seven basic shell structure types. The seventh, known as myostracal layers, is a specialized deposit laid down beneath areas of muscle attachment (Taylor et al 1969) or as the pallial myostracum, as referred to in this paper. Generally, the pallial myostracum lies between layers of differing structural types. Figures 4 and 5 show the pallial myostracum lying between the crossed-lamellar and complex crossed-lamellar structural layers. The pallial myostracum occurs in most, if not all, bivalves (Taylor et al 1969).

Shell Composition

X-ray diffraction studies showed that the shells of D. polymorpha and M. leucophaeta consist entirely of aragonite crystals (Figs. 6a, b and c). Peaks in Figs. 6a, b and c correspond to those characteristic for aragonite crystals. The major peaks for aragonite are found near 3.396, 3.273, 2.700, 2.481, 2.372, 1:977, 1.882 and 1.742_, (Figs. 6a, b, c) as described by Brown (1980). This is in agreement with Kennedy et al (1969) and Taylor et al (1973) who found aragonitic two-layered shells in most other heterodont bivalves, and with Denis (1979) and Archambault-Guezou (1982) who found D. polymorpha to be made up of aragonite crystals. In extant bivalves, the crossed-lamellar and complex crossed-lamellar structures, the pallial myostracal layers, the ligament, and byssus (when calcified) are invariably aragonitic (Kennedy et al 1969).

CONCLUSIONS

1. The most reliable internal diagnostic feature is the presence of an apophysis in *M*. *leucophaeta* and its absence in *D*. *polymorpha*.
2. Slight differences in shell shapes may be enough to distinguish *D*. *polymorpha* from *M*. *leucophaeta*.
3. Shells of both *D*. *polymorpha* and *M*. *leucophaeta* have an outer crossed-lamellar shell structure and an inner complex crossed-lamellar shell structure with a thin, prismatic pallial myostracum between the two layers.
4. Microtubules are more prominent in *M*. *leucophaeta* than in *D*. *polymorpha*.
5. Shells of both *D*. *polymorpha* and *M*. *leucophaeta* are composed entirely of aragonite crystals.

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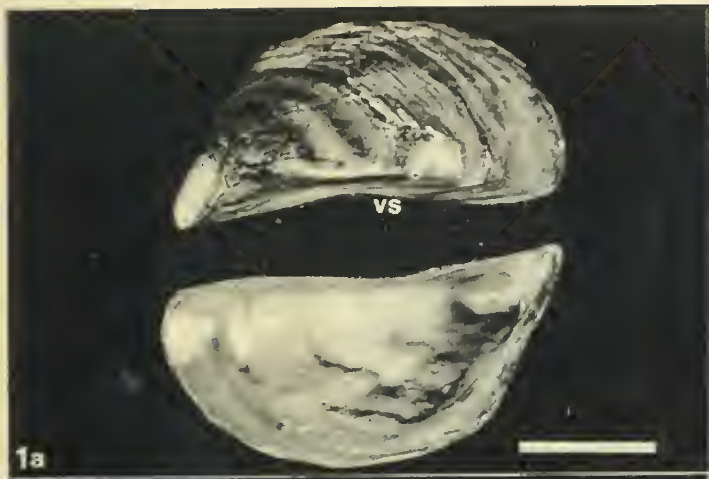
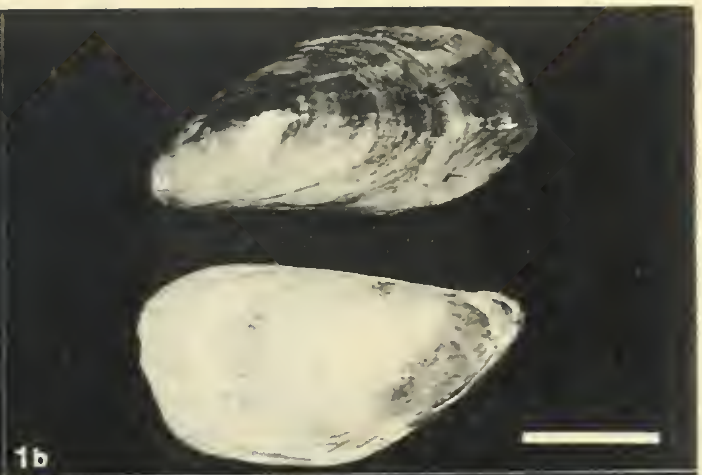
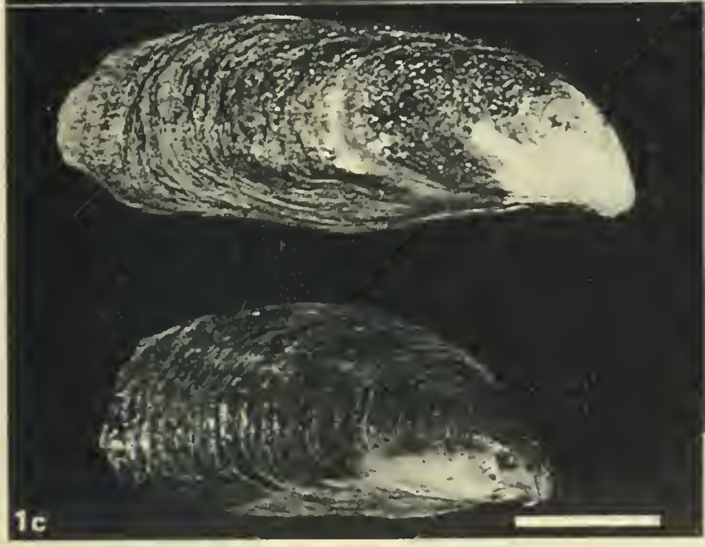






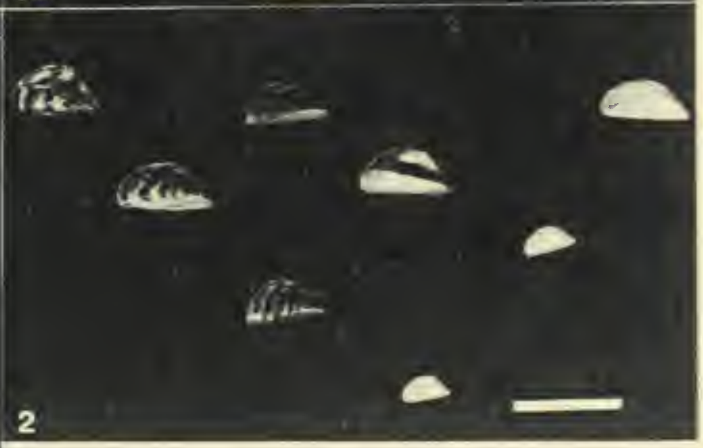
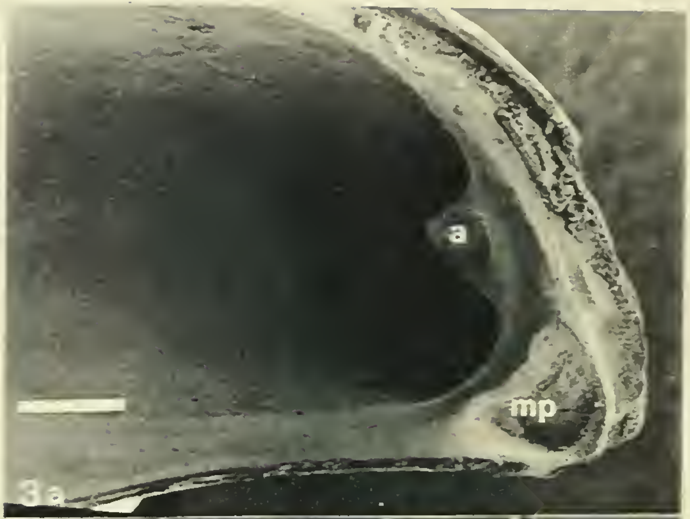


Figure 1(a). Outer view of left valve and inner view of right valve of Dreissena polymorpha. Note the pointed umbone (u), arched and flattened ventral surface (vs), and ventro-lateral shoulder (s). Bar = 0.5 cm

Figure 1(b). Outer view of left valve and inner view of right valve of Mytylopsis leucophaeta. Note the rounded umbone (u) and the absence of both the arched, flattened ventral surface and the ventro-lateral shoulder. Bar = 0.5 cm

Figure 1(c). Right valves of Mytylopsis leucophaeta showing general shell appearance of all dark with no stripes. Shapes and colour patterns may vary. Bar = 0.5 cm

Figure 2. Eight Dreissena polymorpha specimens showing colour and pattern variations within the species. Bar = 1 cm



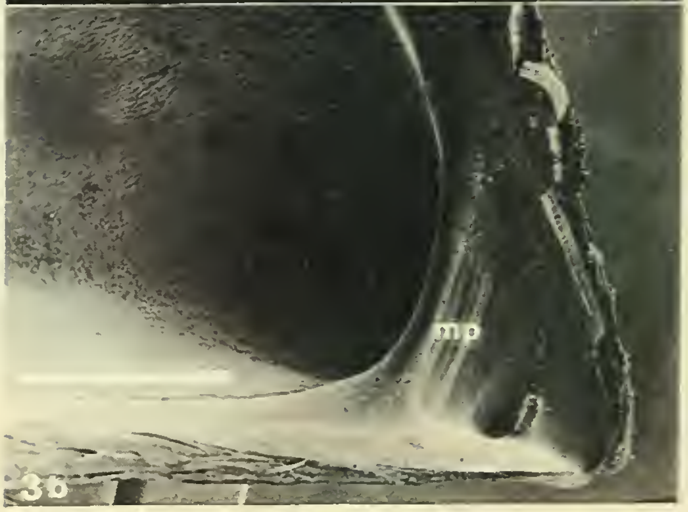
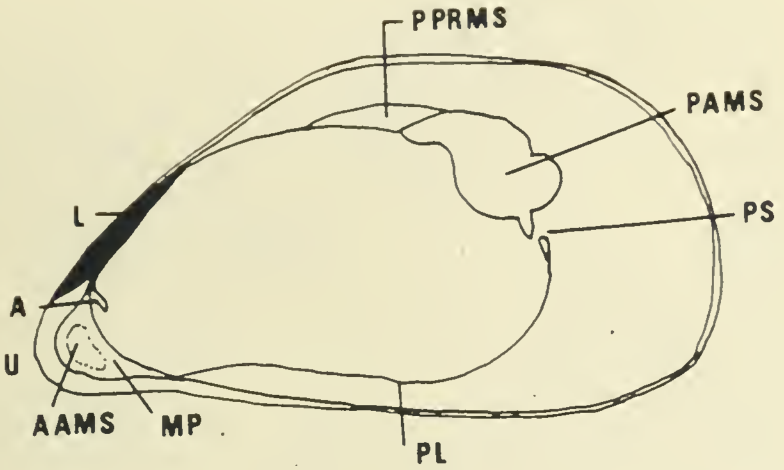


Figure 3(a). Inner view of left valve of Mytylopsis leucophaeta showing anterior region with myophore plate (mp). Note apophysis (a). Bar = 1000 μm

Figure 3(b). Inner view of left valve of Dreissena polymorpha showing anterior region with myophore plate (mp). Note absence of apophysis. Bar = 1000 μm



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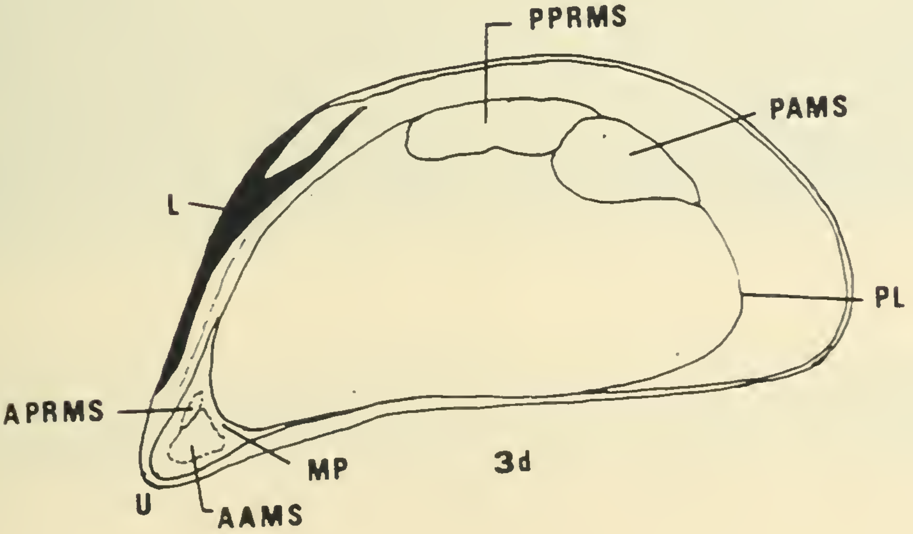

3d

Figure 3(c). Interior view of the right valve of *Mytilopsis leucophaeta* showing the apophysis (A), anterior adductor muscle scar (AAMS), ligament (L), myophore plate (MP), posterior adductor muscle scar (PAMS), posterior pedal-byssal retractor muscle scar (PPRMS), pallial line (PL), pallial sinus (PS) and umbone (U).

Figure 3(d). Interior view of the right valve of *Dreissena polymorpha* showing the anterior adductor muscle scar (AAMS), anterior pedal-retractor muscle scar (APRMS), ligament (L), myophore plate (MP), posterior adductor muscle scar (PAMS), posterior pedal-byssal retractor muscle scar (PPRMS), pallial line (PL) and umbone (U).

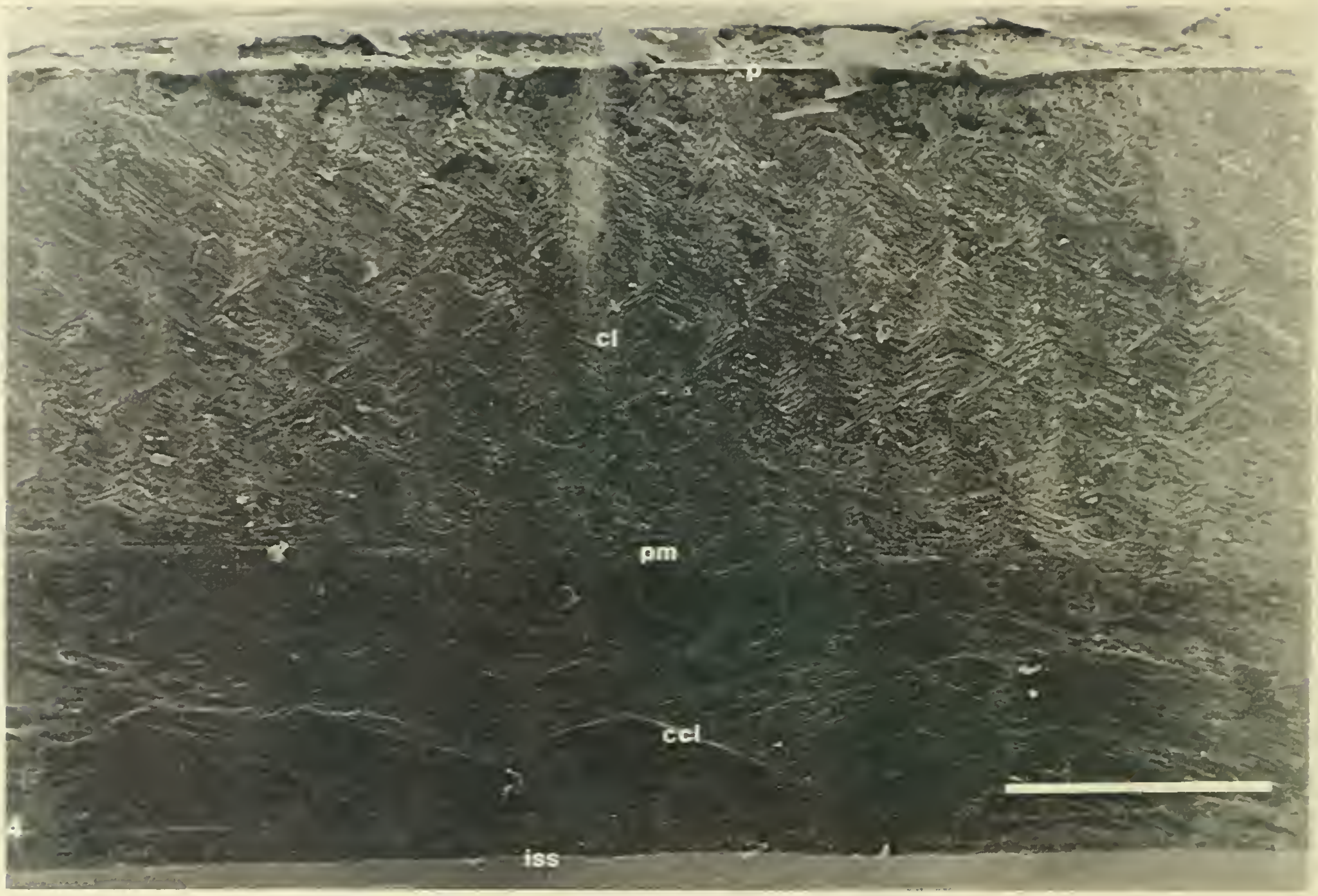

Figure 4. Cross-section through the anterior ventral region of left valve of Dreissena polymorpha. Micrograph shows outer crossed-lamellar (cl) and inner complex crossed-lamellar (ccl) shell structure with a thin, prismatic pallial myostracum (pm) between. Note periostracum (p) and inner shell surface (iss). Bar = 100 μm

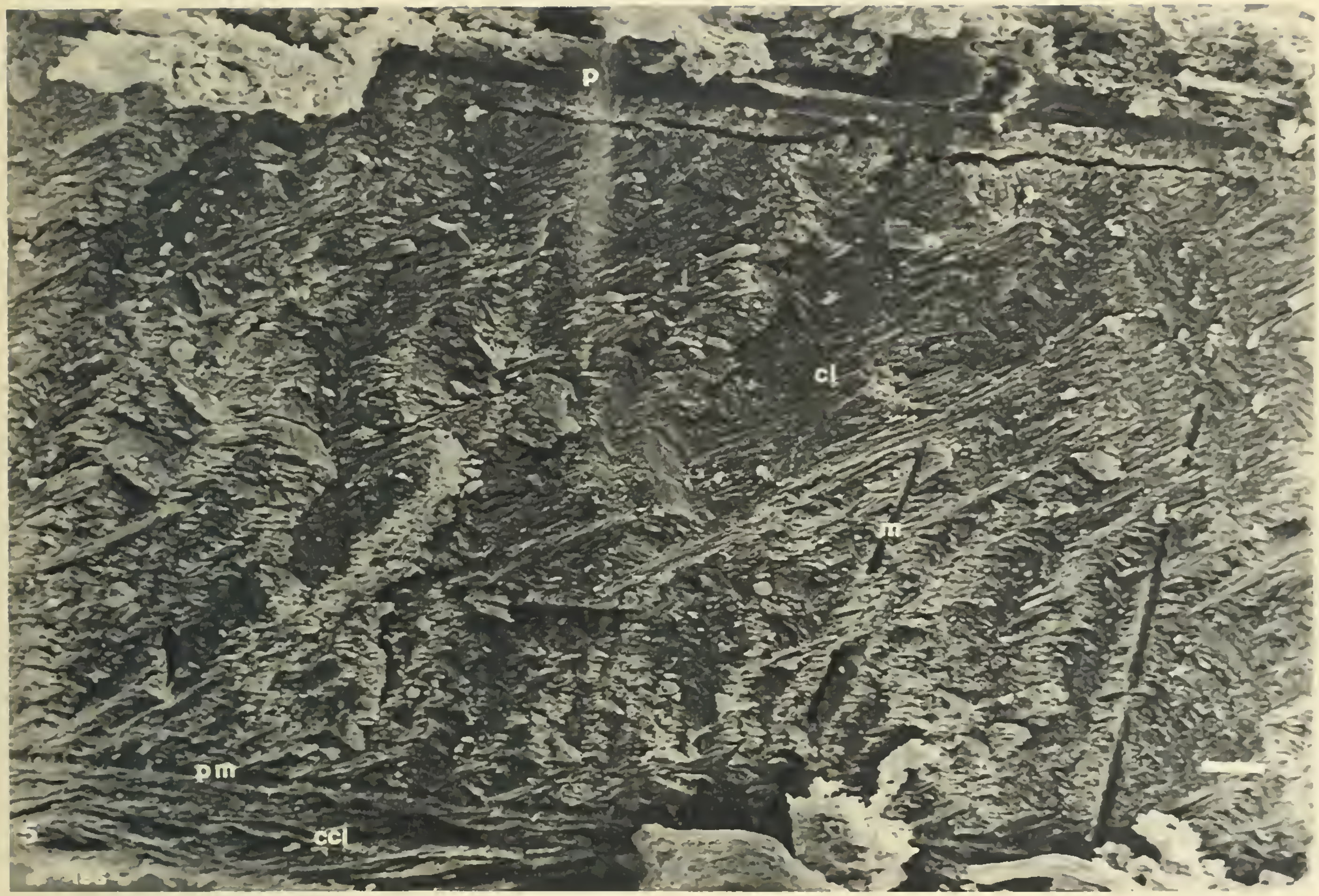

Figure 5. Cross-section through the anterior ventral region of left valve of Mytylopsis leucophaeta. Micrograph shows outer crossed-lamellar (cl) and inner complex crossed-lamellar (ccl) shell structure with a thin, prismatic pallial myostracum (pm) between. Note microtubules (m), periostracum (p), and inner shell surface (iss). Bar = 10 μm

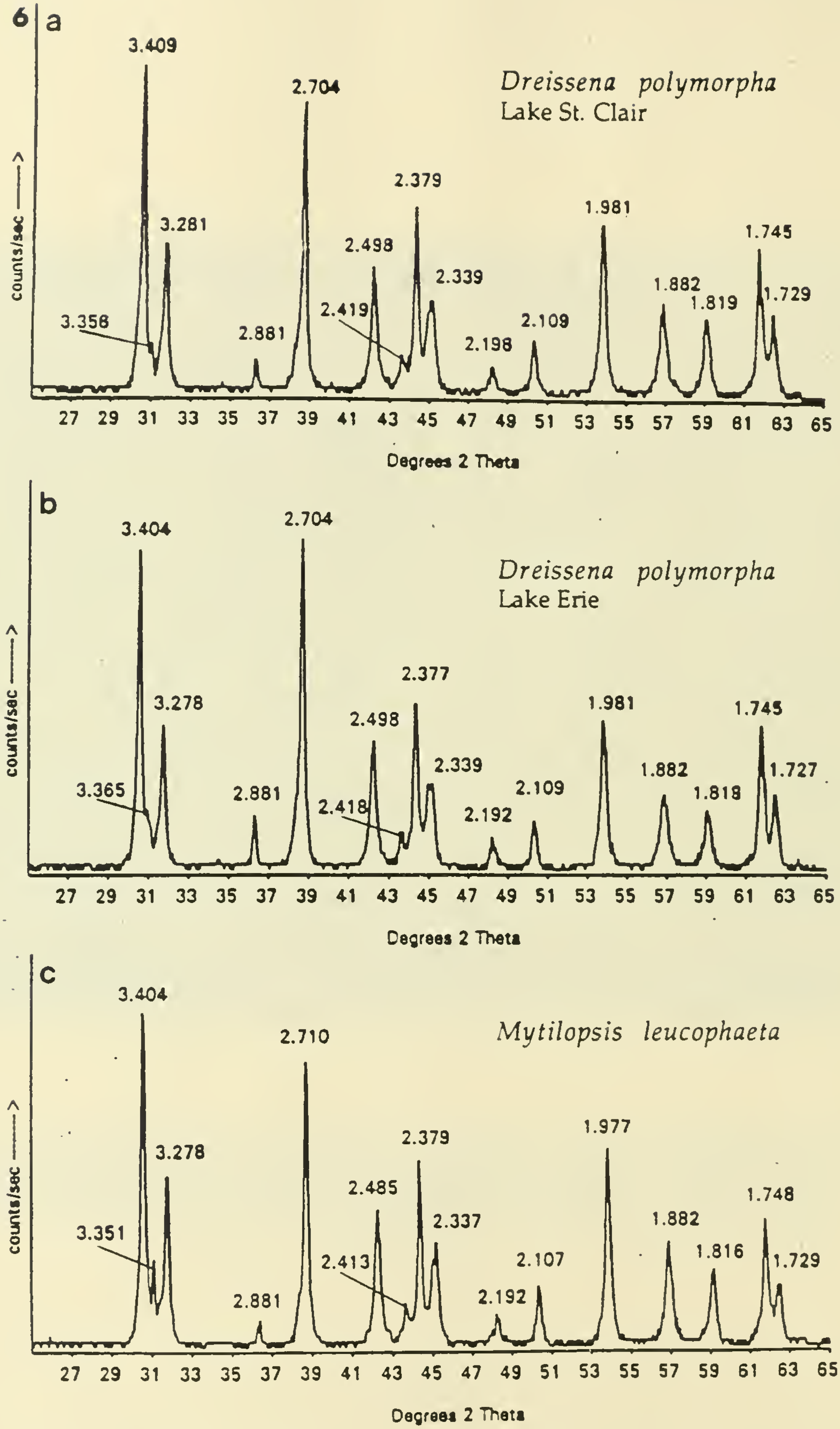

Figure 6. X-ray diffraction graphs of pulverized shell material from *Dreissena polymorpha* at Puce, Lake St. Clair (a), *Dreissena polymorpha* at Port Stanley, Lake Erie (b), and *Mytilopsis leucophaeta* from the Patapsco River, Maryland (c) shells. All peaks correspond to that for aragonite. Y-axis is normalized to highest peak.

LIFE HISTORY AND DEMOGRAPHICS OF POPULATIONS OF THE ZEBRA MUSSEL (DREISSENA POLYMORPHA) IN LAKE ST. CLAIR AND LAKE ERIE

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Guelph, Ontario N1G 2W1.

SUMMARY

The zebra mussel, Dreissena polymorpha, was probably introduced into the Great Lakes probably in the fall of 1985, from the ballast water of freighters originating in Europe. First discovered in Lake St. Clair in June of 1988, it is now found throughout all the Great Lakes and has begun to invade inland water systems in Canada and the United States. The life history and demographics of the zebra mussel population at Puce, Ontario, along the southwest shore of Lake St. Clair has been studied, and comparisons made to other populations in the Great Lakes and Europe. Adult zebra mussels from the Puce area have a life span of about 1 - 2 years and rapid growth rates of over 1.5 cm per year. Maximum shell lengths average 1.5 - 2.0 cm, with standing crops ranging from a low of 695.m^{-2} to over 200,000.m^{-2}. Fertilization is external and veligers appear to require 3 - 5 weeks before settlement occurs. In 1990 the first appearance of free swimming veliger larvae at Puce was observed on June 20 at a water temperature of 21°C, but in 1991, larvae first appeared mid-May at 15°C. In 1990 veliger numbers peaked in the first week of July at 162,000 m^{-3} and disappeared from the water by mid-October. One peak in veliger abundance occurred in 1990 while two peaks occurred in 1989. Within Lake St. Clair there are annual variations in the time of first appearance of larvae, number of peaks per year, abundance and settling periods.

INTRODUCTION

The zebra mussel, Dreissena polymorpha, is an exotic bivalve species native to the Black, Azov and Caspian Seas (Morton 1969b, Stanczykowska 1977). First discovered in the 1700's the zebra mussel colonized most of Europe's freshwater systems over the course of 200 years (Morton 1969c). Zebra mussels were introduced into the Great Lakes from the ballast water of freighters originating in Europe. Zebra mussels were first discovered in Lake St. Clair in June of 1988, and age structure analysis of the shells suggests the mussels first colonized the lake in 1985 (Hebert et al 1989).

The free-swimming larvae of the zebra mussel enable them to spread rapidly downstream at a rate of up to 250 km per year (Mackie 1991). The zebra mussel's attachment to boat hulls ensures its eventual spread upstream and inland. These dispersal mechanisms and the zebra mussel's high fecundity have resulted in the rapid expansion of the zebra mussel throughout the Great Lakes and into inland water systems in Canada and the United States.

The life cycle of the zebra mussel is illustrated in Figure 1. Zebra mussels mature in their second year of life (Morton 1969a, Stanczykowska 1977). During the first year of sexual maturity a female can release 30,000 eggs, with an increase to 40,000 eggs in the third and fourth years (Karpevich 1955, Katchanova 1961). An adult female, with a life span of 4-5 years, can produce up to 150,000 eggs in its lifetime (Stanczykowska 1977). Fertilization is external and upon hatching the eggs turn into free-swimming veliger larvae. The veliger stage or straight-hinged form is characterized by the "D" shaped veliger, with lengths ranging from 53 to 112 μm. The post-veliger stage is referred to as either the umbonal or veliconcha form and is characterized by the formation of the umbone, with lengths ranging from 112 to 200 μm. In the settling or pediveliger stage, the umbone is more pronounced and the veliger lengths range from 180 μm and up. At this stage the veligers lose their ability to swim, fall from the water column and attach to hard substrates and begin the adult life cycle (Mackie et al 1989).

Zebra mussels posses numerous strong byssal threads that enable it to attach to almost any hard substrate, such as rocks, water intake pipes of industries, boats, navigational aids, native unionid bivalves, as well as other zebra mussels. This has resulted in severe economic problems for "users" of Great Lakes water and poses a threat to the survival of natural inhabitants of the Great Lakes (Lewandowski 1976, Mackie et al 1989, Mackie 1991).

The objectives of this study are: (1) to compare the seasonal and annual variations in abundance, birth periods, numbers of recruitment events, and durations of occurrences of zebra mussel veliger larvae from Lake St. Clair and Lake Erie; (2) to determine variations in the growth rates of individuals and of populations in Lake St. Clair and Lake Erie and of the life spans of adults; and (3) to determine the standing crops of adult zebra mussels from Lake St. Clair, at Puce.

Mackie (1991) has already given some basic descriptions of the D. polymorpha population in Lake St. Clair, including its allometry of growth, length-weight relationship, life cycle, and the average individual growth rate and are not repeated here. All these studies were supported by funding from the Ontario Ministry of Environment, Project No. 443G.

MATERIALS AND METHODS

A study site on Lake St. Clair and one on Lake Erie were chosen, based on the facts that zebra mussels were first found in Lake St. Clair and their veliger larvae are known to be rapidly dispersed downstream by currents. Puce is located on the southwest shore of Lake St. Clair, with Port Stanley along the north shore of Lake Erie's central basin (Fig. 2). Both are near-shore, wade-in sites.

Sampling was done every two weeks from April - September 1990, then once a month to February 1991. Zebra mussels from Puce were also collected during the summer and fall of 1989. Water samples were collected each trip for measurements of pH, temperature and alkalinity to characterize the habitats. The variation in each was similar and well within the ranges normally seen in Lake St. Clair and Lake Erie, pH varying from 8.0 to 8.2 (mean = 8.1) and 8.0 - 8.3 (mean 8.1), temperature from 10.2°C in November to 28.5°C in mid-summer (mean 22.1°C) and 2.5°C in December to 24.5°C in mid-summer (mean = 20.2°C), and alkalinity from 93 to 112 mg $CaCO_3$/L (mean 106 mg $CaCO_3$/L) and 95 to 118 mg $CaCO_3$/L (mean 112 mg $CaCO_3$/L), respectively.

Six replicates of veliger samples were collected using a 12-L Schindlar-Patalas plankton trap in water less than 1 m in depth. All plankton samples were preserved with 70% ethanol and observed under a dissecting scope to determine zebra mussel veliger larvae densities and size frequency throughout the season.

Even though zebra mussels attach to almost any hard substrate, for this study rocks were used as sampling units. Anywhere from 1 to 5 rocks were collected each trip in water no more than 1m in depth. For each rock all the zebra mussels were scraped off using a scalpel and blade. The zebra mussels were preserved in 70% ethanol, allowed to dry, counted and measured using a digitizing tablet to determine length frequency distributions and growth rates. The surface areas of the exposed rocks available to the zebra mussels for colonization were determined using a tinfoil wrapping technique and densities per m^2 of zebra mussels determined.

RESULTS

Seasonal Abundances and Length-Frequency Distributions of Veliger Larvae in Lake St. Clair and Lake Erie.

At Puce, veligers were first observed in the water column June 20th, at a water temperature of 21 °C. The peak number of veliger larvae was observed July 4th at a peak of 162,000 veligers m^{-3}. The numbers of veligers steadily decreased after the peak, and disappeared from the water column by mid-October (Fig. 3).

At Port Stanley, veligers were first observed in the water column July 17th, at a water temperature of 20 °C, but at very low numbers. The peak abundance occurred August 1st at 941,000 veligers m^{-3}. The numbers of veligers then steadily decreased through to the end of September. A second smaller peak may have occurred in September but veligers were last observed in the water column in early November (Fig. 4).

From May to September 1990 at Puce, the veligers were primarily of the D-form with lengths ranging from 67.5 to 112.5 μm. Few veligers from the veliconcha and pediveliger stages, with lengths $\geq$ 112.5 μm, were observed (Fig. 5). From July to October 1990 at Port Stanley, the veligers were primarily of the veliconcha stage, with lengths from 112.5 μm to 200 μm and the pediveliger stage, with lengths of over 200 μm (Fig. 6).

Seasonal Length-Frequency Distribution of Adult Mussels at Puce, Lake St. Clair.

Figures 7-15 represent the length-frequency data for zebra mussels collected from September 1989 to February 1991, for Lake St. Clair, at Puce. The data suggests that the D. polymorpha population at Puce was composed of one to possibly three cohorts. The dominant cohort from May 10, 1989 (Fig. 7a), with a mean length of around 4.5 μm, can be seen to increase in size through to July 1989 (Figs. 7c, 8a and b), with a new cohort, the young-of-the-year for 1989, making an appearance in late July 1989 (Fig. 8b). As this new cohort increases in number and size, the dominant cohort appears to decrease in number and disappear by the end of September 1989 (Fig. 9a).

The same type of trend can be seen for the 1990 data. The new cohort from 1989 becomes the dominant cohort for 1990 (Figs. 9b and c). Again, you see this dominant cohort increasing in number and size through into the middle of July 1990 (Fig. 12b), with a new 1990 cohort making an appearance by the middle of July 1990 (Fig. 12b). By the middle of July 1990, when the new cohort for 1990 appears, the dominant cohort for that year begins to disappear (Fig. 12b). The new cohort for 1990 can be seen to increase in size and number from August through to December 1990 (Figs. 12c and 13-15). The largest zebra mussel found was 2.95 cm on August 15, 1990 (Fig. 13a). The life span for the zebra mussels appears to be only one year.

Apparently there was a settlement event between December and February in Lake St. Clair because a new cohort with a mean length near 3 mm had appeared in the Feb. 8th sample (Fig. 15b). This is somewhat surprizing since there was only about 1.5 mm of adult shell growth from September to December (Figs. 14a to 15a). However, it is possible that some adults in the largest cohort, with a mean size between 15 and 18 mm, may have been contributing to the veliger pool throughout the fall but larval development rates were reduced because of low temperature and settlement did not occur until sometime between December and February.

Growth Rates and Standing Crops of Adult Zebra Mussels in Lake St. Clair.

From May to November 1989 and 1990, the growth rate of the adult zebra mussels is very similar, with growth rates of about 1.5 to 2 cm per year (Fig. 16).

Seasonal variations in the mean number of zebra mussels per m of hard substrate from Lake St. Clair at Puce varied seasonally by nearly three orders of magnitude, from a low of $695.m^{-2}$ in the spring of 1990 to a high of $283{,}101.m^{-2}$ by the middle of August 1990 (Fig. 17). Although the variations in standing crops of adult mussels on rocks was very large, the average standing crop appeared to increase by more than one order of magnitude, from about 12,500 m^{-2} in 1989 to about 155,000 m^{-2} in 1990.

DISCUSSION

While the life history of D. polymorpha is similar in many lakes, variations are common (Mackie et al 1989). Table 1 is a summary of the veliger data for the Puce and Port Stanley sites. There was a difference of about one month between the two sites for the first appearance of veliger larvae. The water temperature at which veligers were first observed was very similar for both sites, around 20 - 21 °C. This temperature is well above the reproductive threshold temperature range, of 14 - 16 °C, that is reported in the European literature (Morton 1969, Stanczykowska 1977, Galperina 1978). In 1991 veligers first appeared at Puce the middle of May at a water temperature of 15 °C (Mackie pers. com.). Perhaps the absolute temperature per se is not as important in reproduction as the number of degree days at or above a specific temperature. However, with the bimonthly sampling protocol, veligers may actually have appeared sometime between the sampling trips when the water temperature was lower, and was not observed with this sampling protocol.

For both sites the veliger abundance peaks occurred about three weeks after the veligers were first observed in the water column, with Port Stanley having a much higher veliger abundance peak, $941{,}000.m^{-3}$ vs $162{,}000.m^{-3}$ for Puce, the veligers remaining in the water column longer at the Port Stanley site. Port Stanley had a second, but much smaller, veliger abundance peak in 1990, while Puce had only one abundance peak in 1990. However, both Port Stanley and Puce had two abundance peaks in 1989 (Mackie, pers. comm.). The number of veliger abundance peaks per season indicates the number of spawning periods throughout the season.

In Figure 5, only the D-form veligers are well represented in the veliger length frequency data for Puce. Most likely the pediveligers, of lengths ≥ 180μm, had already settled out and were not represented in this graph. In contrast, in Figure 6, there are few D-form veligers, and more veliconcha

(112.5 - 180/200 μm), and pediveligers, ($\geq$ 180/200 μm) represented in the veliger length frequency data for Port Stanley. The D-form veligers most likely had already developed into veliconchas with the large number of pediveligers being a result of either the resuspension of juveniles in the water column or veligers that were growing to larger sizes before settling out. The bimonthly sampling protocol did not always provide enough data to clearly show the trend involving zebra mussel veliger development, from their release through to the settling stage.

Earlier studies suggest the onset of settlement of veligers occur about three to five weeks after the first appearance of the veliger larvae, with the time of peak settlement occurring about six weeks after the veliger abundance peaks (Pathy and Mackie, 1991). This explains why the new cohort, or young of the year, at Puce was seen on July 16, 1990 (Fig. 12b), about one month after the veligers were first observed in the water column on June 20, 1990 (Fig. 3, Table 1). It also explains why the time of peak settlement occurs around the middle to end of August 1990 (Fig. 3a and b), at least six weeks after the time of peak veliger abundance at Puce (Fig. 3, Table 1).

In both 1989 and 1990, the dominant cohort of the year appears to disappear by August 1990 (Figs. 12c, 13a and b). This decline and/or disappearance of the larger size class may be caused by: (1) a natural die off of the older zebra mussels; (2) the detachment of the byssal threads resulting in the disappearance of the larger size class. In either case, the remains of the byssal threads should be present, indicating the earlier presence of the larger size class.

Zebra mussels from Puce averaged around 1.5 - 2 cm in length, with the largest zebra mussel from Puce measuring 2.95 cm, sampled August 15, 1990 (Fig. 13a). Morton (1969b) describes the European length limit as 5.1 cm, with dead shells of 5 cm in length being found. The largest zebra mussel obtained from Lake St.Clair was sampled from near Grosse Point, Michigan in July 1991 and was 3.9 cm in length (Gillis, pers. comm.).

The apparent life span of only one year is considerably shorter than the European literature suggests. Depending on the area, life spans of 3 - 9 years have been reported in Europe (Mackie et al 1989), demonstrating that the life span of the zebra mussel is highly variable among lakes.

The increase in number of zebra mussels per m on August 15, 1990 (Fig. 17), corresponds to the settlement of the new cohort for the year, that can be seen in the length frequency graphs for August 1 and 15, 1990, (Figs. 12c and 13a). European densities of zebra mussels are high with numbers from 5,000 to a high of 114,000 zebra mussels m^{-2}, being reported (Mackie et al 1989). These numbers fall well within the range of 695 to 283,101 zebra mussels m^{-2} for Lake St. Clair, at Puce.

CONCLUSIONS

1. Between sites there are annual variations in the time of first appearance of veliger larvae, number of peaks per year, abundance and settling periods.
2. To successfully determine the seasonal variations in abundance and length frequency distributions of zebra mussel veliger larvae, a sampling protocol of at least once a week is required, while biweekly sampling is preferred.
3. The 1989 and 1990 zebra mussel population at Puce was dominated by a single cohort with less than 10% of the population containing mussels from a second cohort.
4. The zebra mussel population at Puce appears to have a life span of only one year and a growth rate of 1 - 1.5 cm a season.
5. Standing crops of zebra mussels per m^2 of hard substrate varied seasonally by nearly three orders of magnitude, and ranged from a low of 695 in the spring of 1990, to a high of 283,101 after settlement in August of 1990.

ACKNOWLEDGEMENTS

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Table 1. Summary of 1990 veliger data for Lake St. Clair, at Puce and Lake Erie, at Port Stanley.

	Puce	**Port Stanley**
1st Appearance of Veliger Larvae	20 June 1990	17 July 1990
Water Temp °C	21.0	20.0
Date of Peak Veliger Abundance	4 July 1990	1 August 1990
Peak Number of Veliger Larvae M^{-3}	162,000	941,000
Last Appearance of Veligers	mid-October 1990	5 November 1990

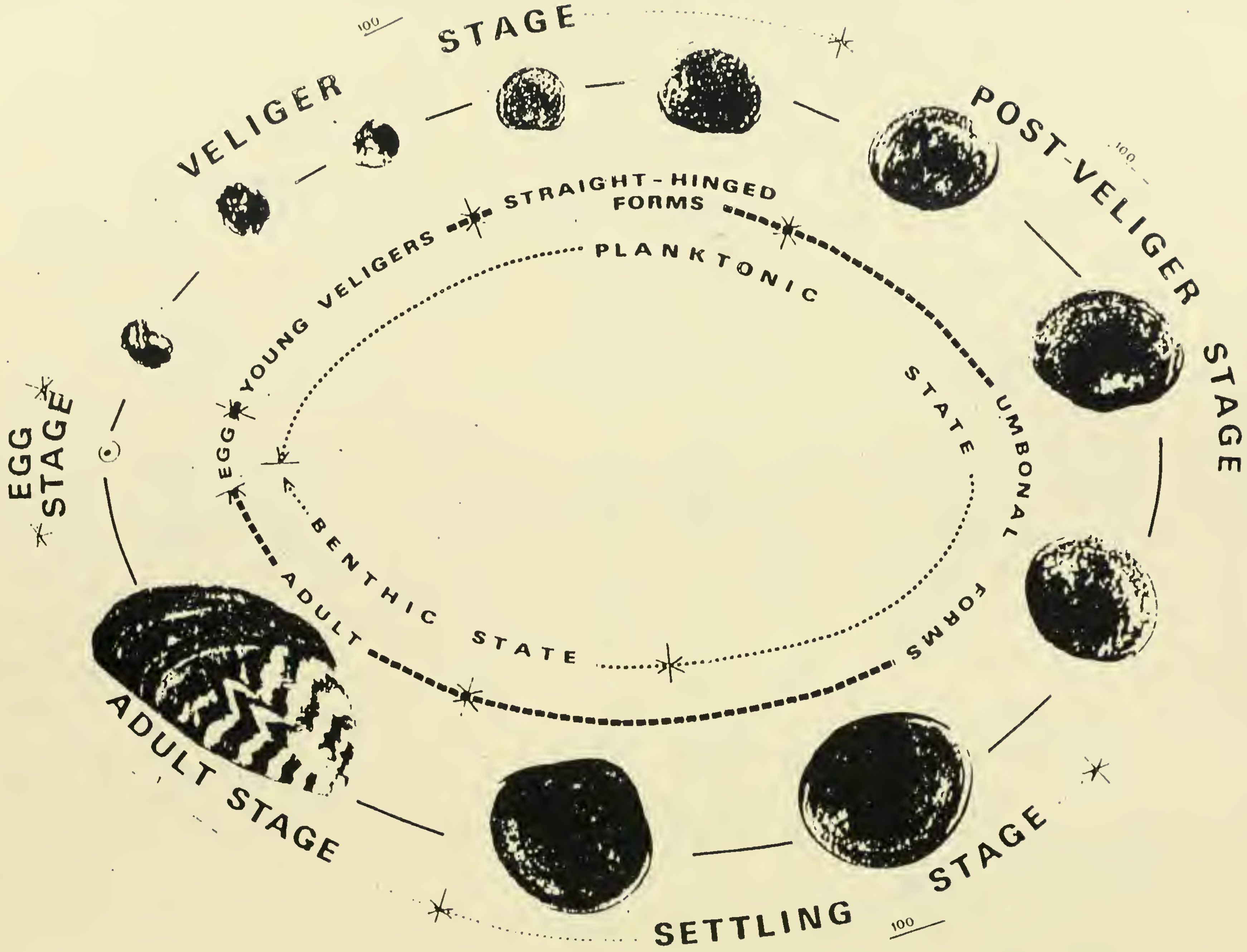


Figure 1. Life cycle of *Dreissena polymorpha*.

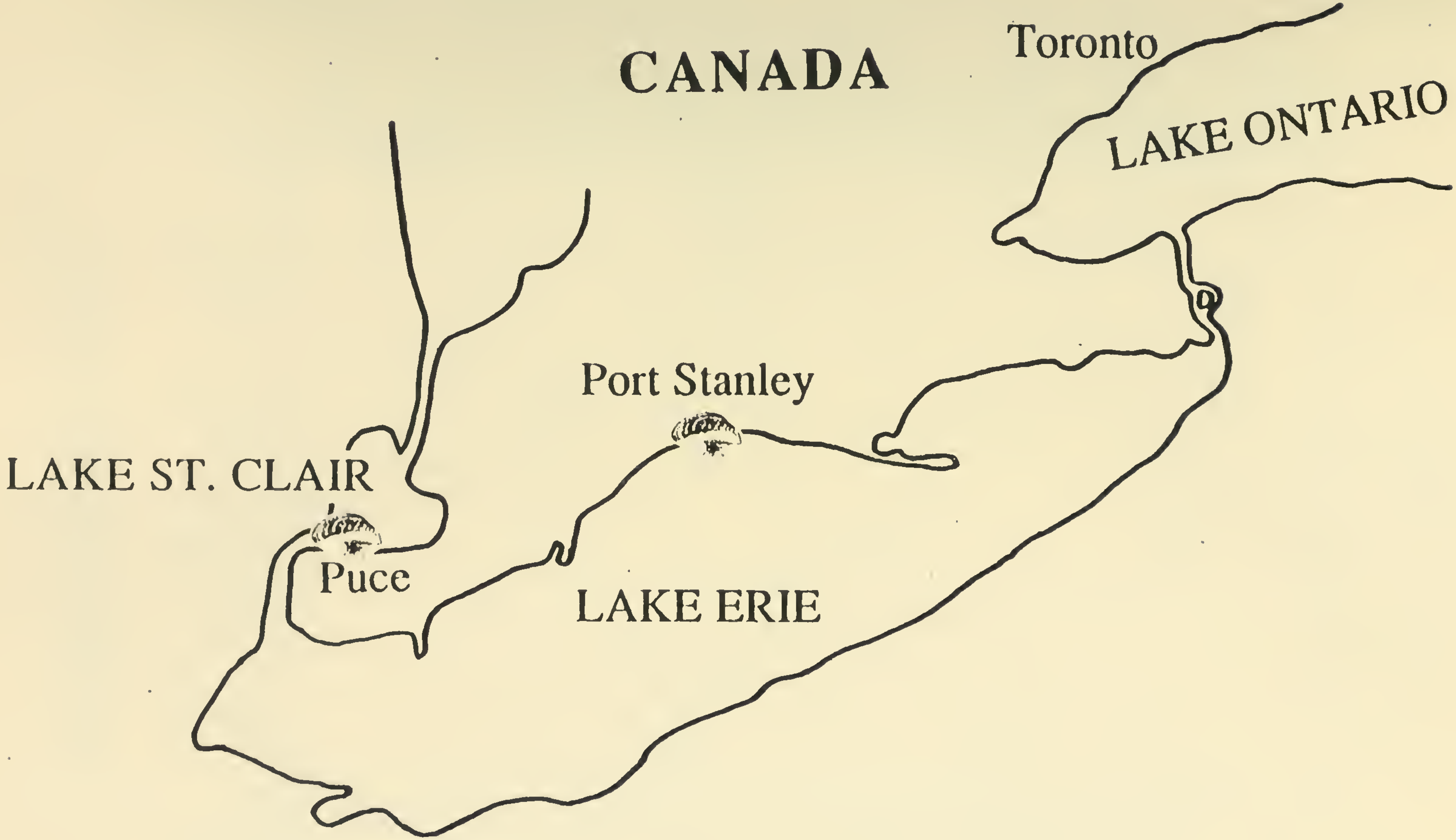


Figure 2. Lake St. Clair and Lake Erie, Ontario, sampling sites for 1989 and 1990.

Figure 3. Seasonal abundance of veliger larvae in relation to temperature in Lake St. Clair, at Puce, 1990.

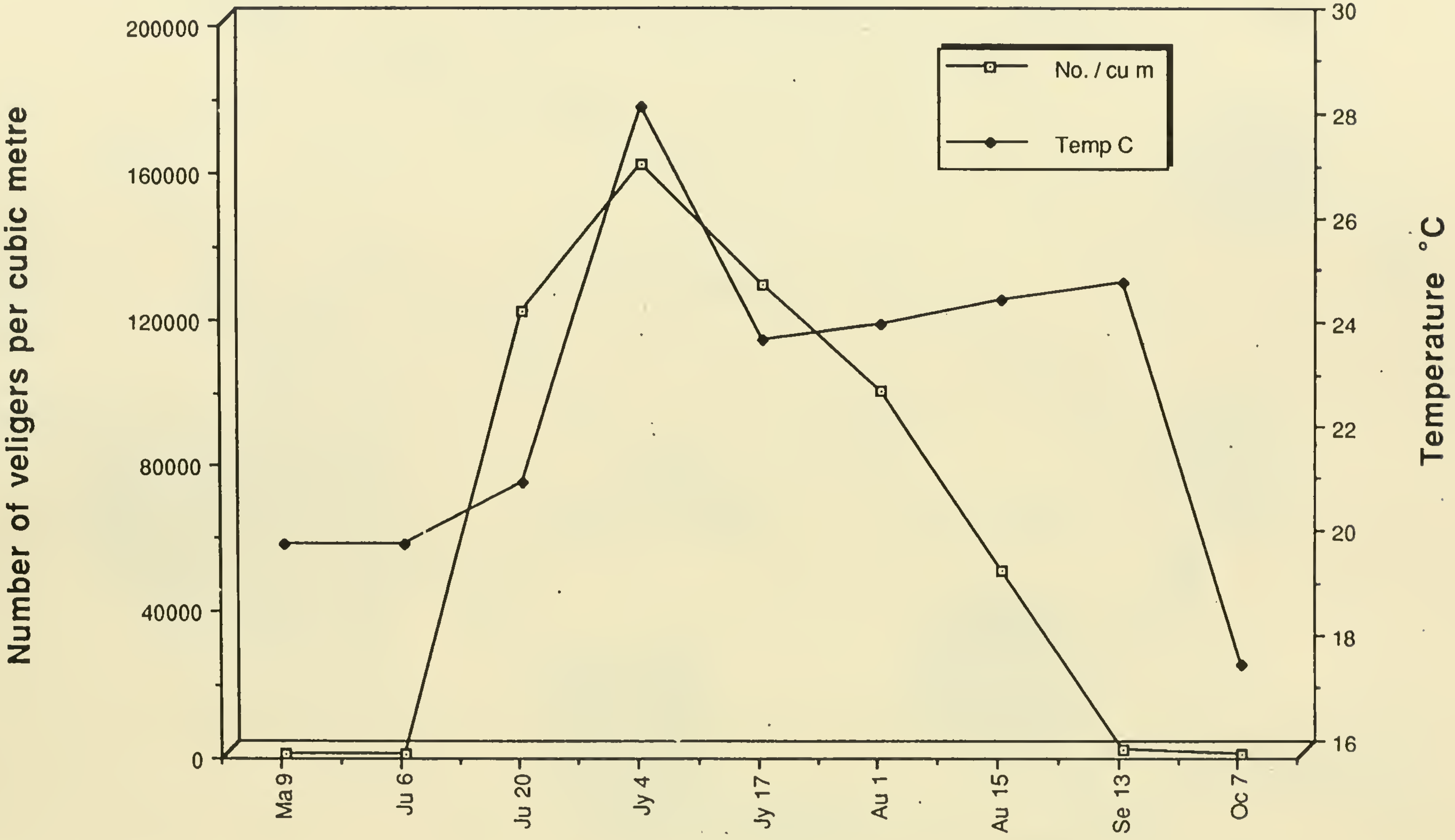


Figure 4. Seasonal abundance of veliger larvae in relation to temperature in Lake Erie, at Port Stanley, 1990.

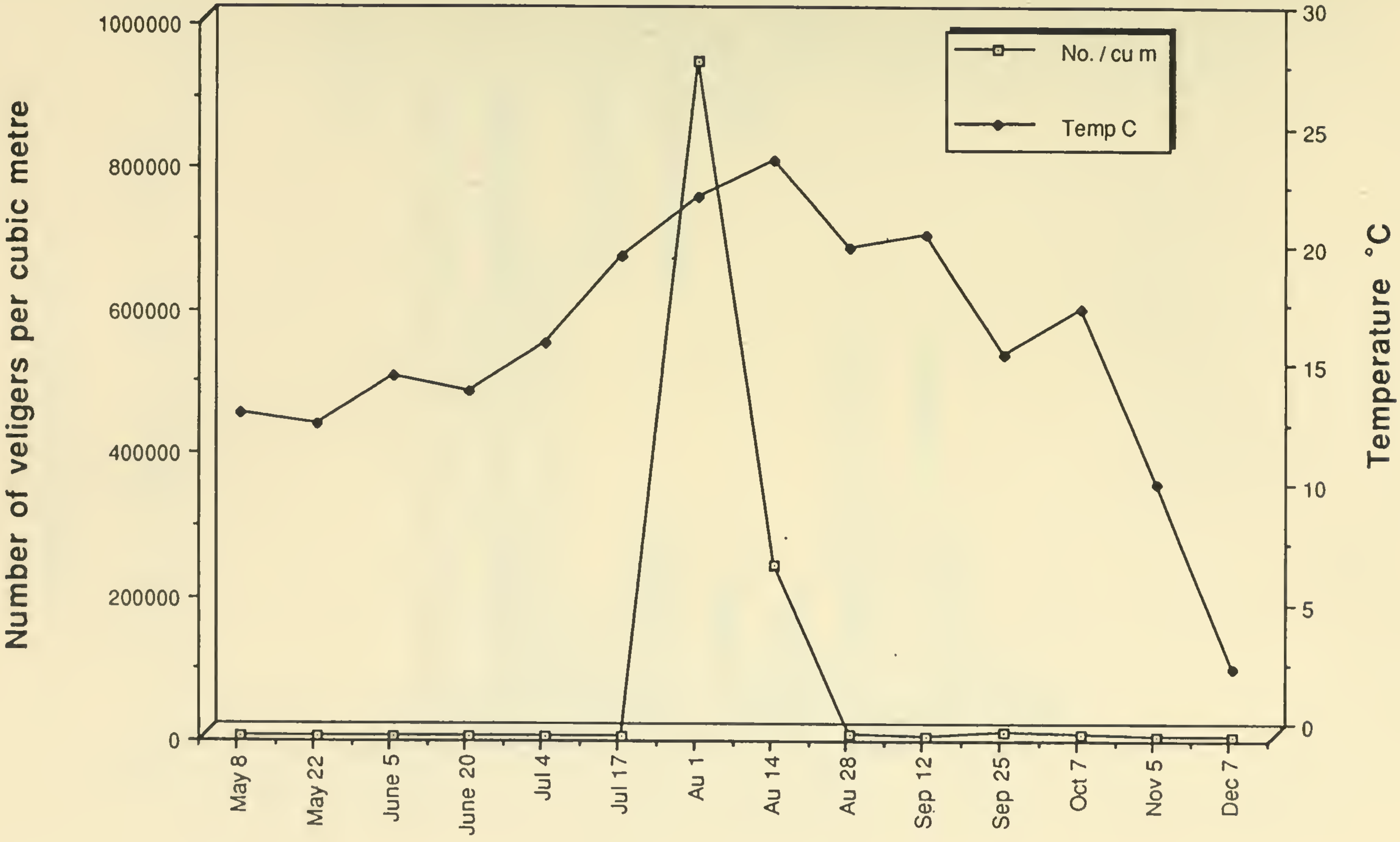


Figure 5. Length frequency distribution of veliger larvae in Lake St. Clair, at Puce, 1990.

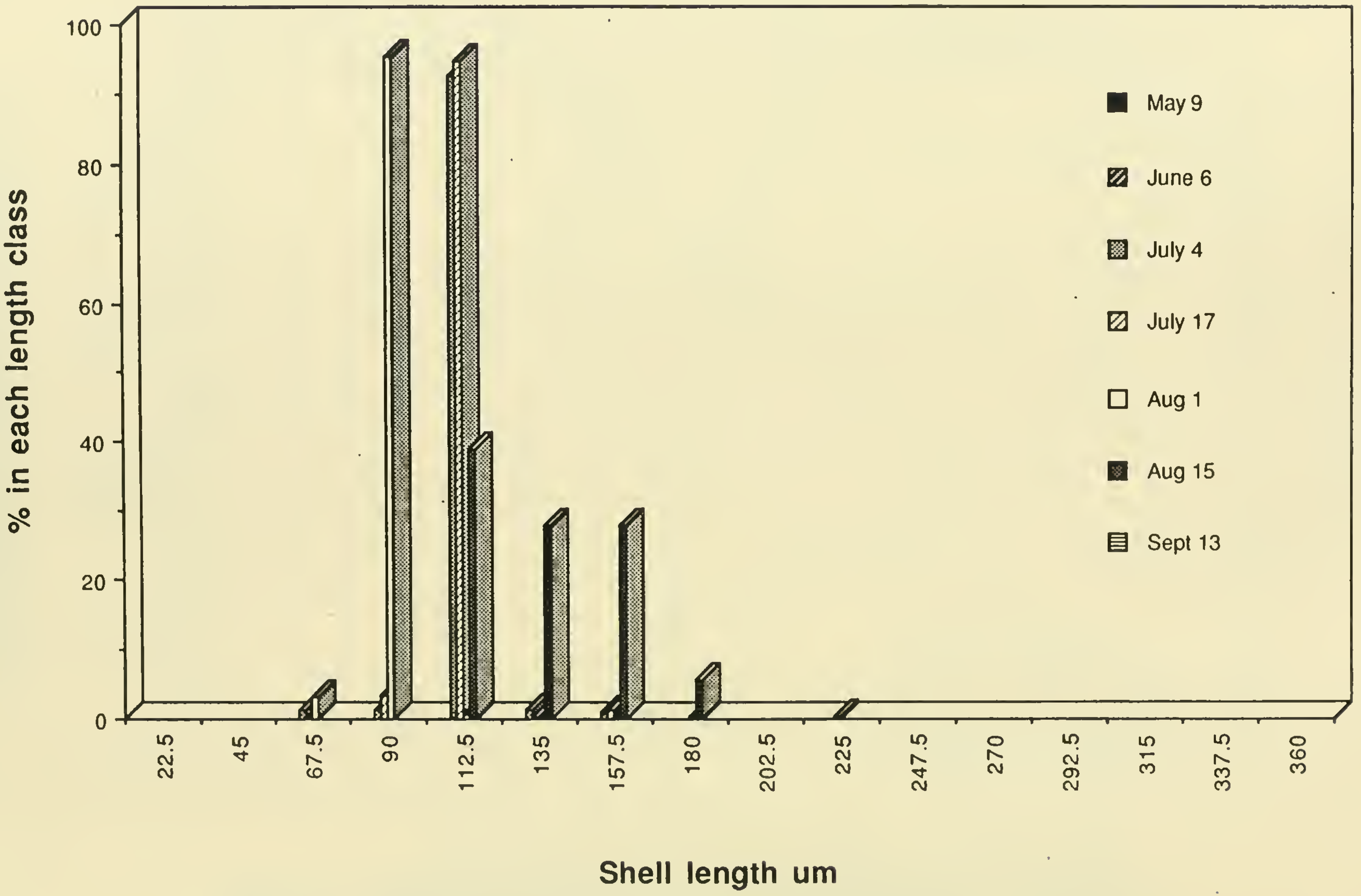
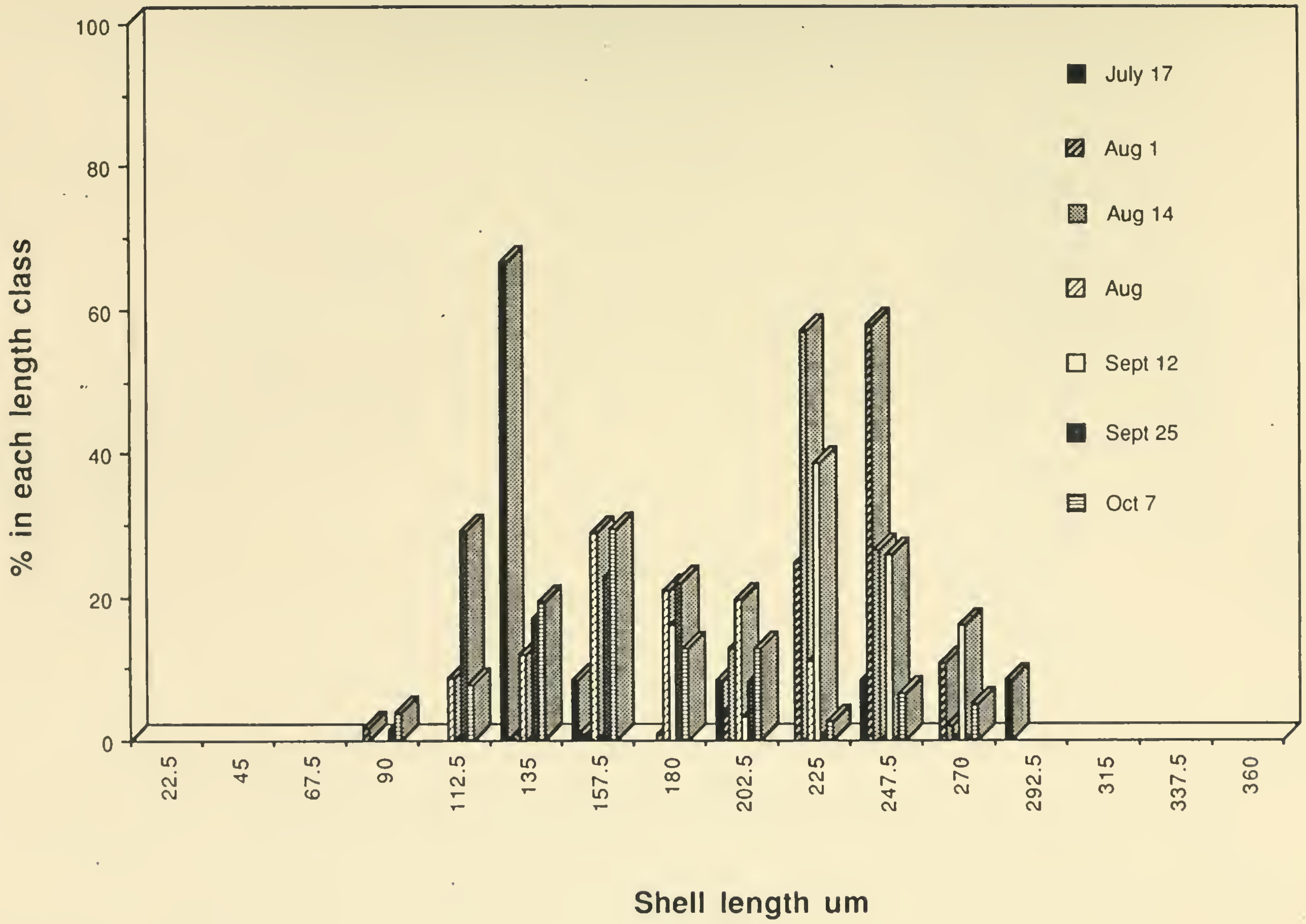


Figure 6. Length frequency distribution of veliger larvae in Lake Erie, at Port Stanley, 1990.



Figures 7 - 15.

Length-frequency histograms for *Dreissena polymorpha* from Lake St. Clair, at Puce, 1989 - 1991.

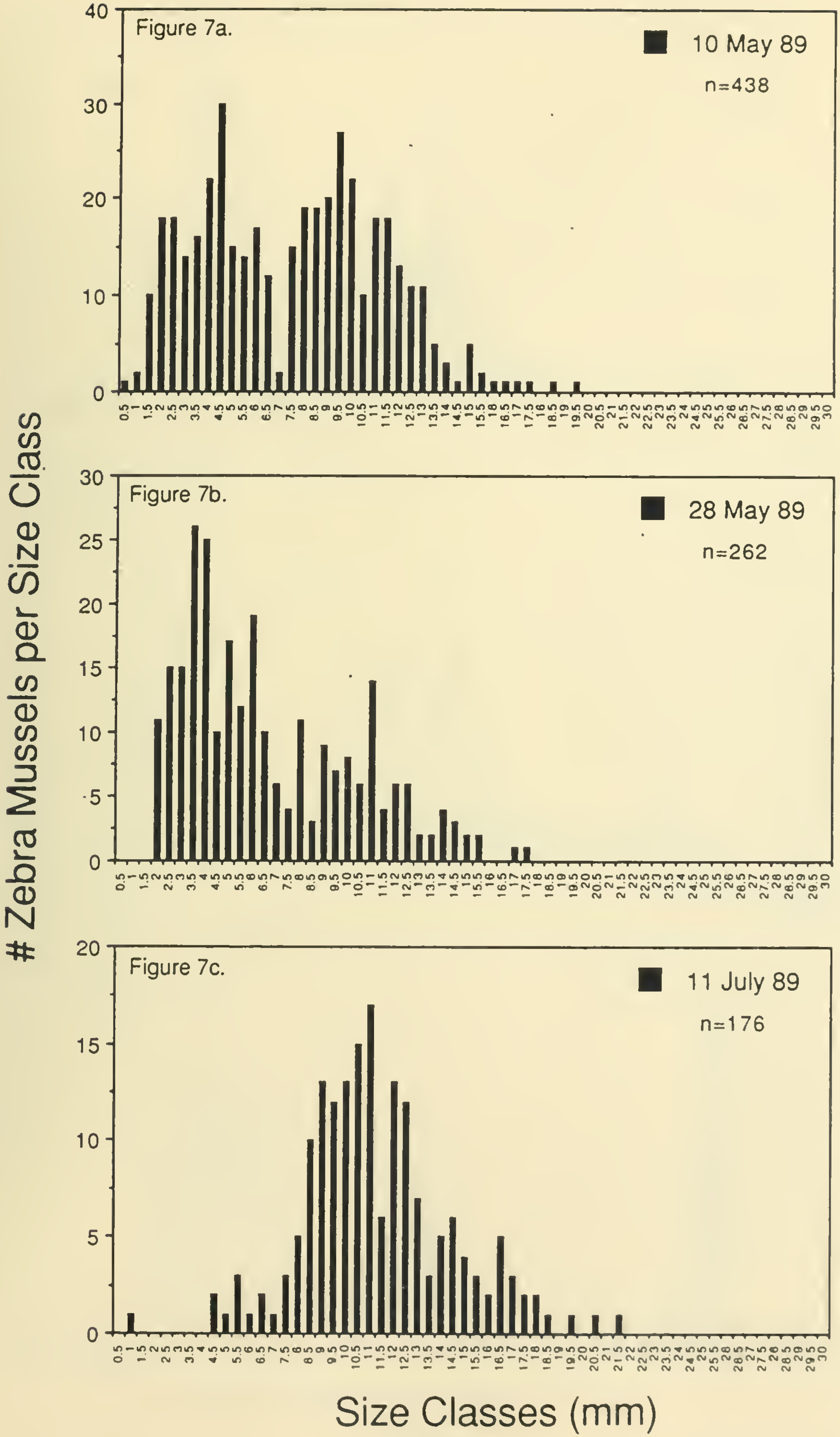


Figure 7a.
■ 10 May 89
n=438
0
10
20
30
40
Figure 7b.
■ 28 May 89
n=262
0
5
10
15
20
25
30
Figure 7c.
■ 11 July 89
n=176
0
5
10
15
20
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
# Zebra Mussels per Size Class
Size Classes (mm)

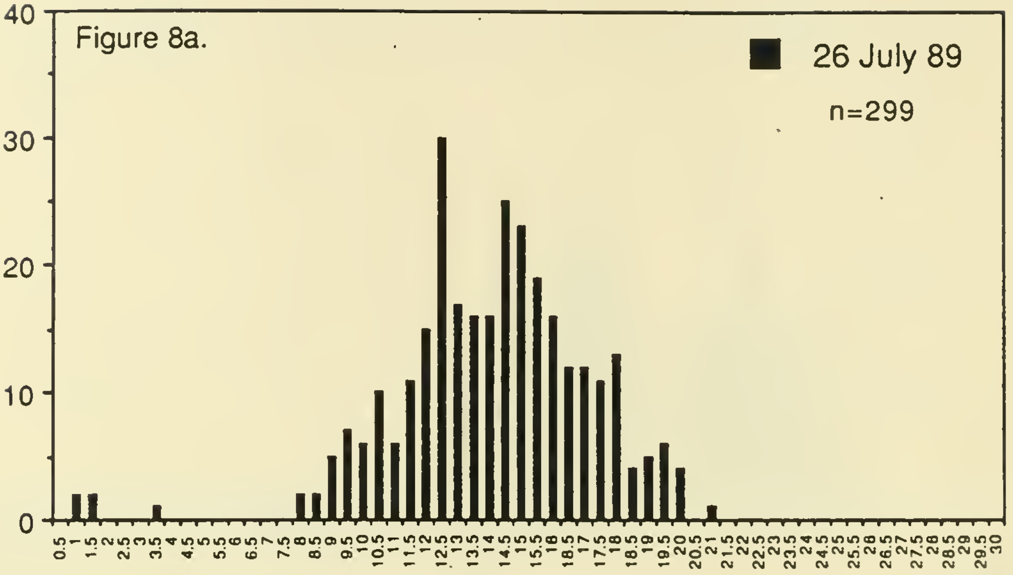


Figure 8a.
26 July 89
n=299
0
10
20
30
40
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30

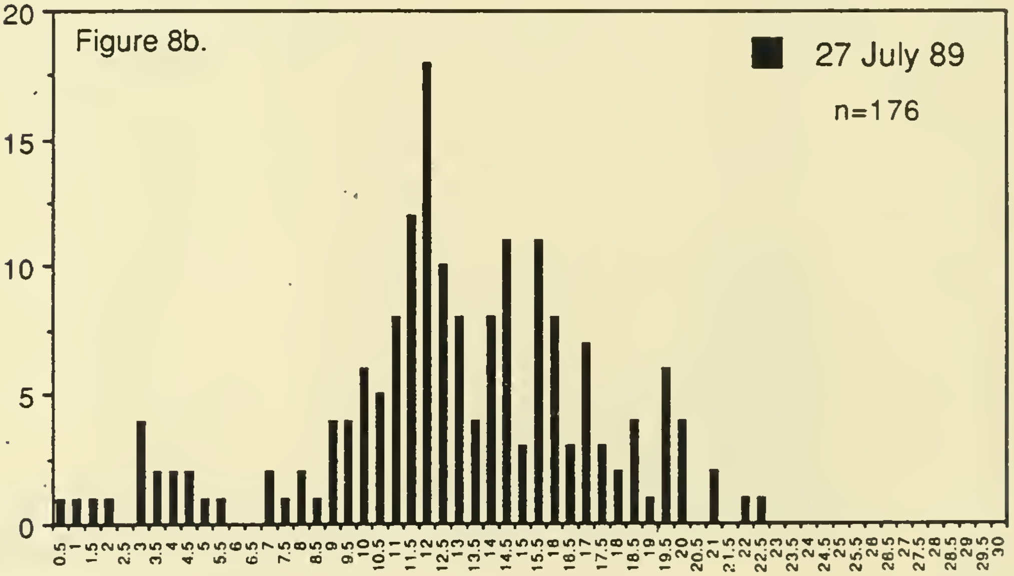


Figure 8b.
27 July 89
n=176
0
5
10
15
20
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
# Zebra Mussels per Size Class

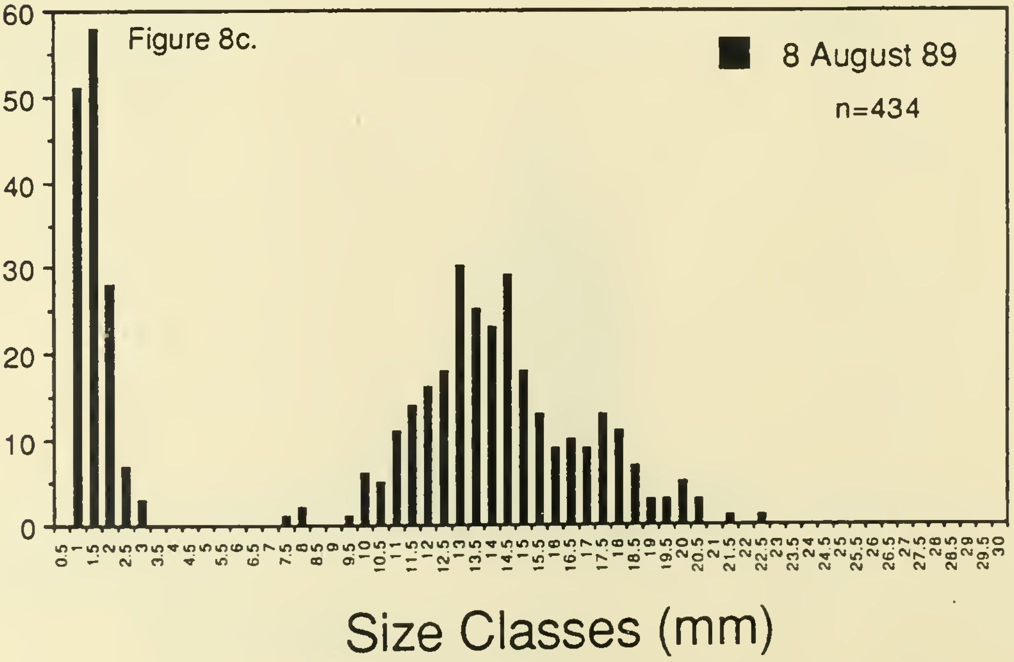


Figure 8c.
8 August 89
n=434
0
10
20
30
40
50
60
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
Size Classes (mm)

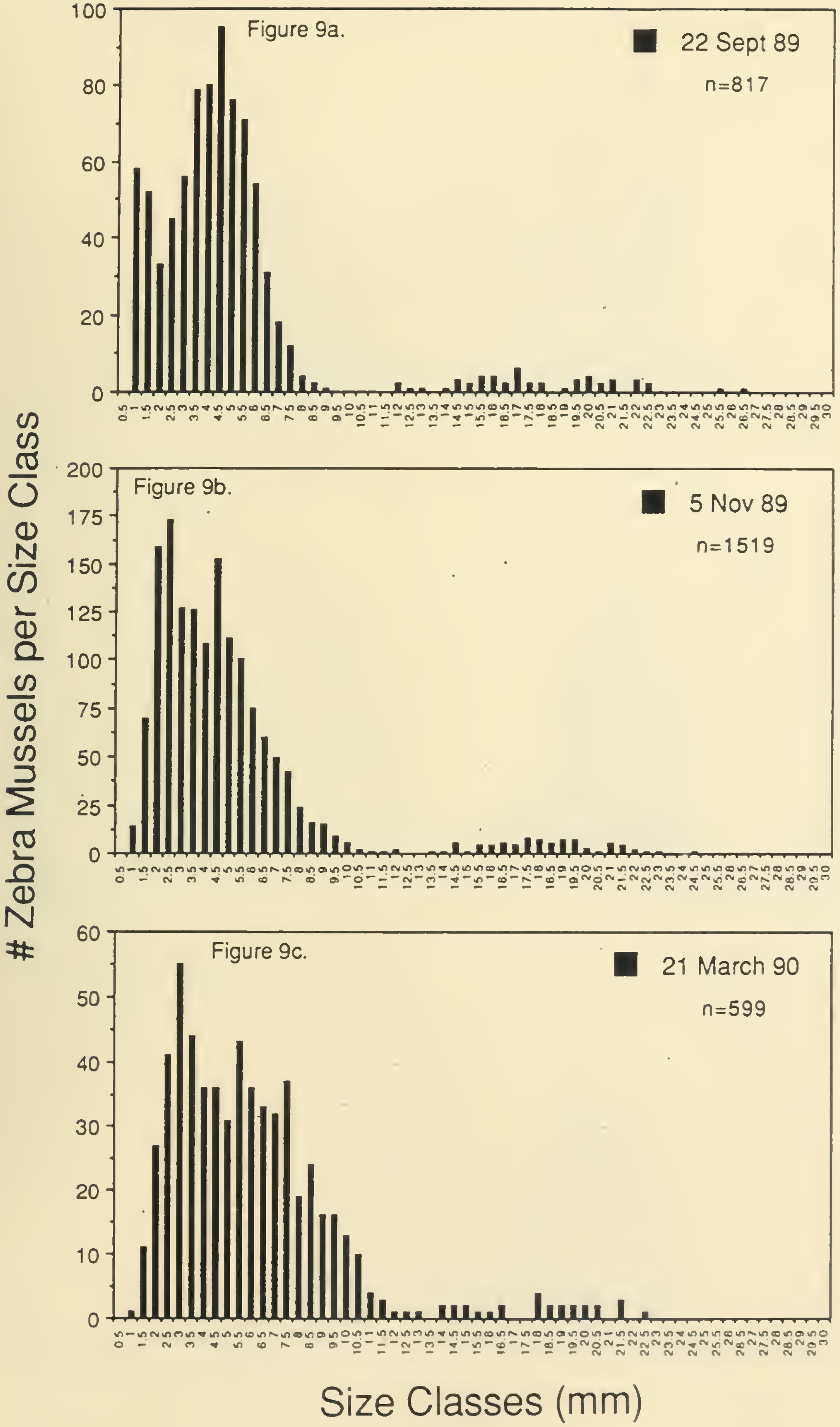


Figure 9a.
22 Sept 89
n=817
0
20
40
60
80
100
Figure 9b.
5 Nov 89
n=1519
0
25
50
75
100
125
150
175
200
Figure 9c.
21 March 90
n=599
0
10
20
30
40
50
60
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
# Zebra Mussels per Size Class
Size Classes (mm)

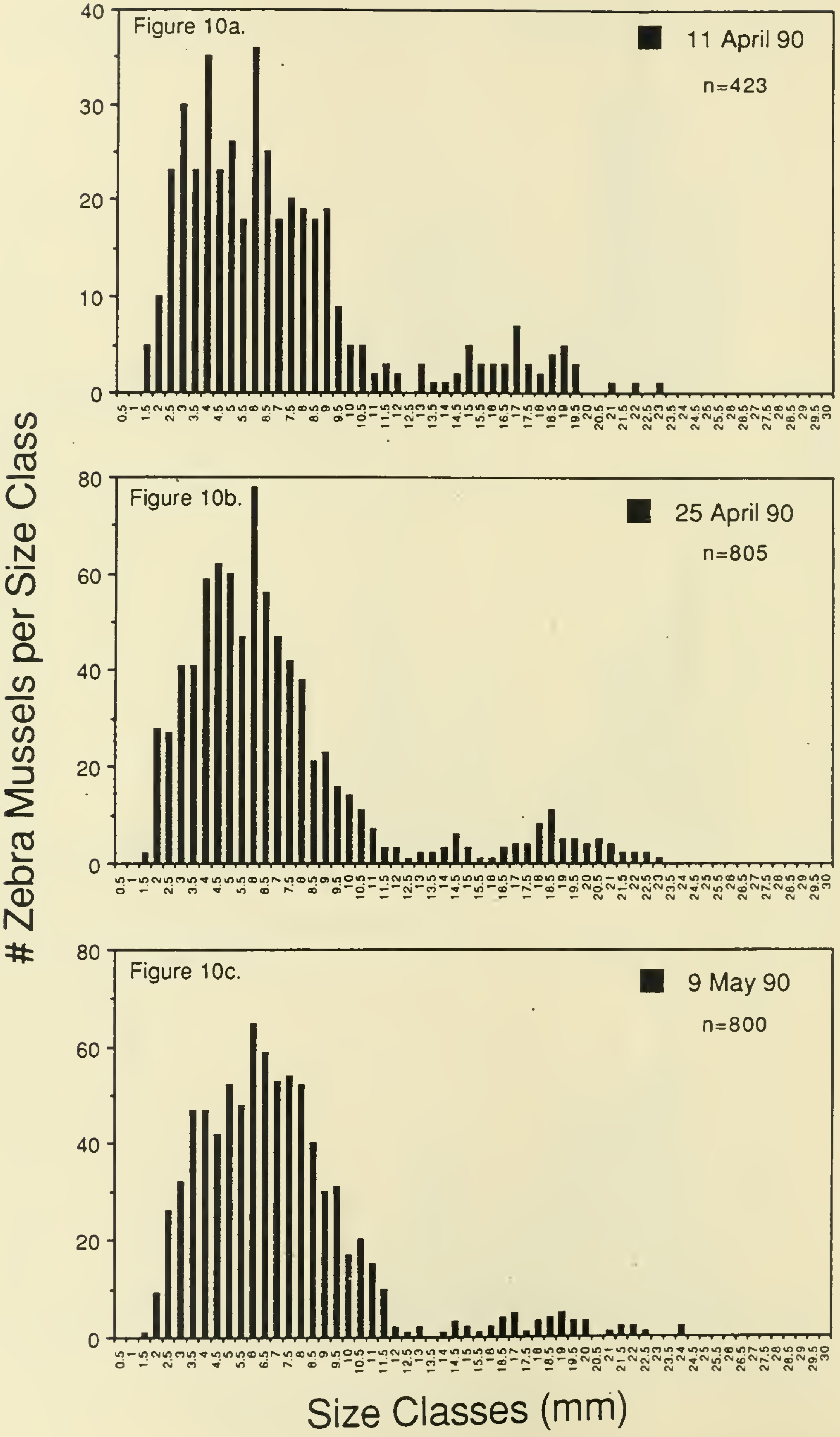


Figure 10a.

Figure 10b.

Figure 10c.

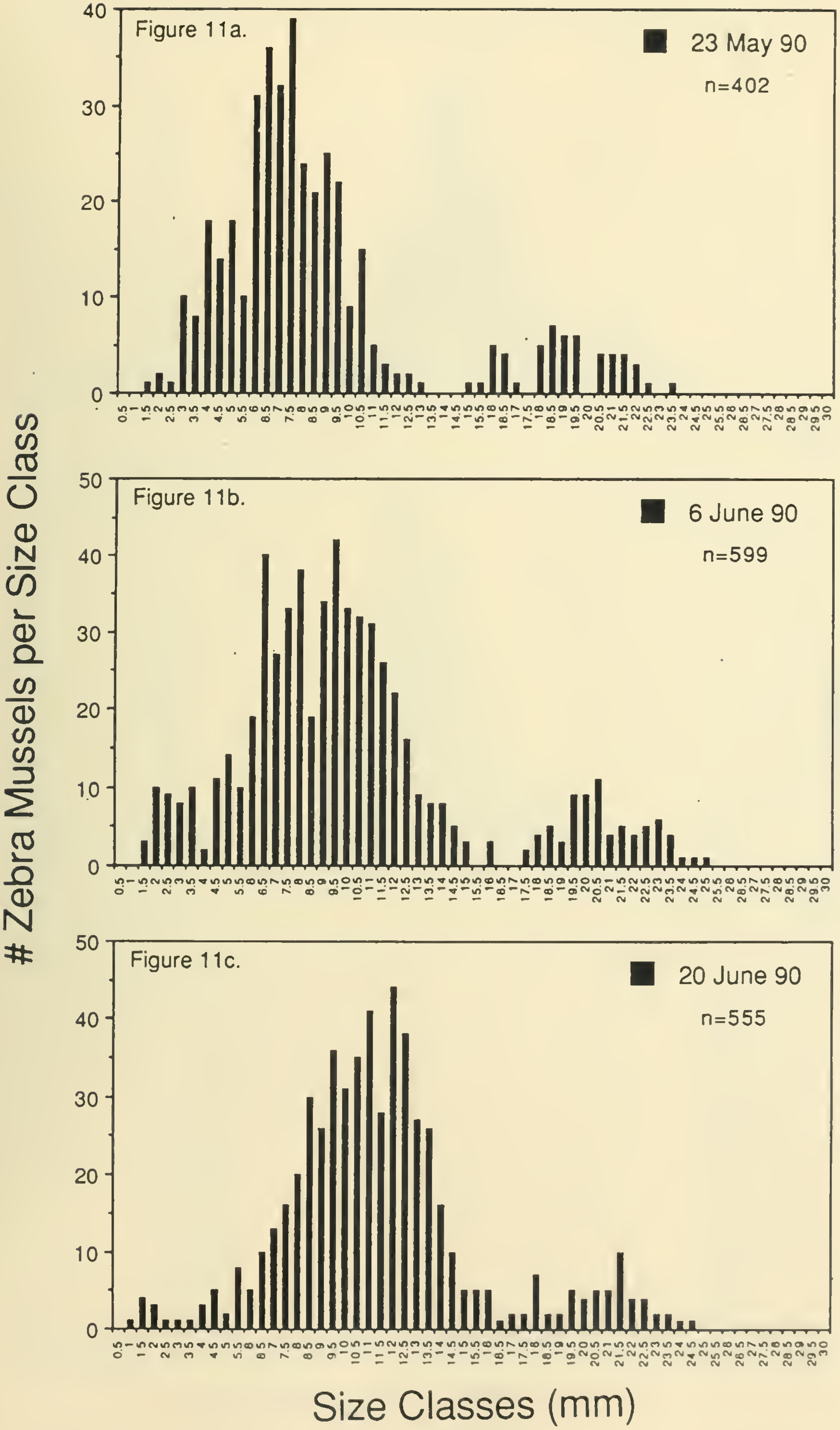


Figure 11a.
23 May 90
n=402
0
10
20
30
40
Figure 11b.
6 June 90
n=599
0
10
20
30
40
50
Figure 11c.
20 June 90
n=555
0
10
20
30
40
50
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
# Zebra Mussels per Size Class
Size Classes (mm)

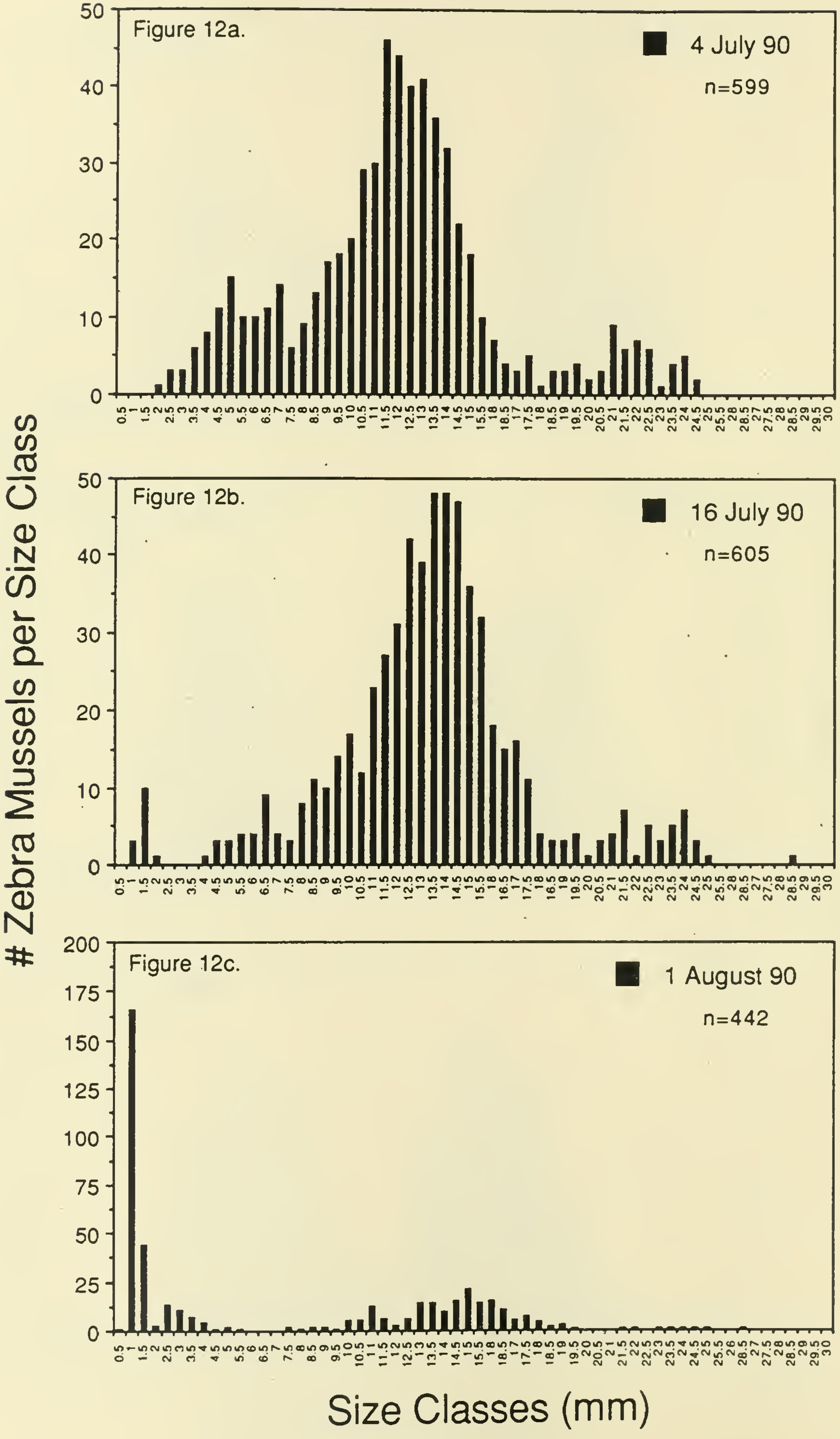


Figure 12a.
4 July 90
n=599
Figure 12b.
16 July 90
n=605
Figure 12c.
1 August 90
n=442
0
10
20
30
40
50
0
25
50
75
100
125
150
175
200
# Zebra Mussels per Size Class
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
Size Classes (mm)

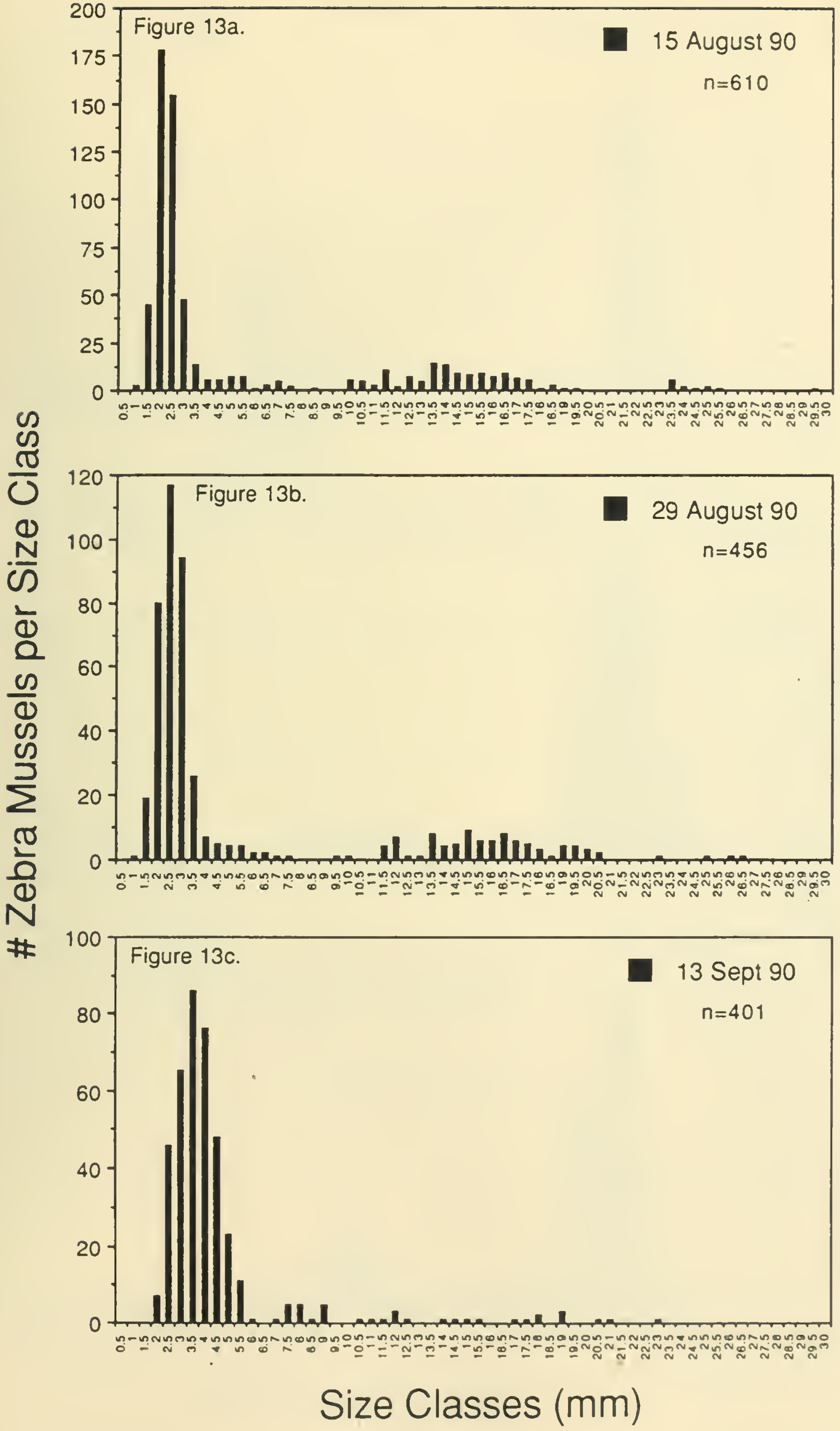


Figure 13a.
15 August 90
n=610
200
175
150
125
100
75
50
25
0
Figure 13b.
29 August 90
n=456
120
100
80
60
40
20
0
Figure 13c.
13 Sept 90
n=401
100
80
60
40
20
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 16 16.5 17 17.5 18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30
# Zebra Mussels per Size Class
Size Classes (mm)

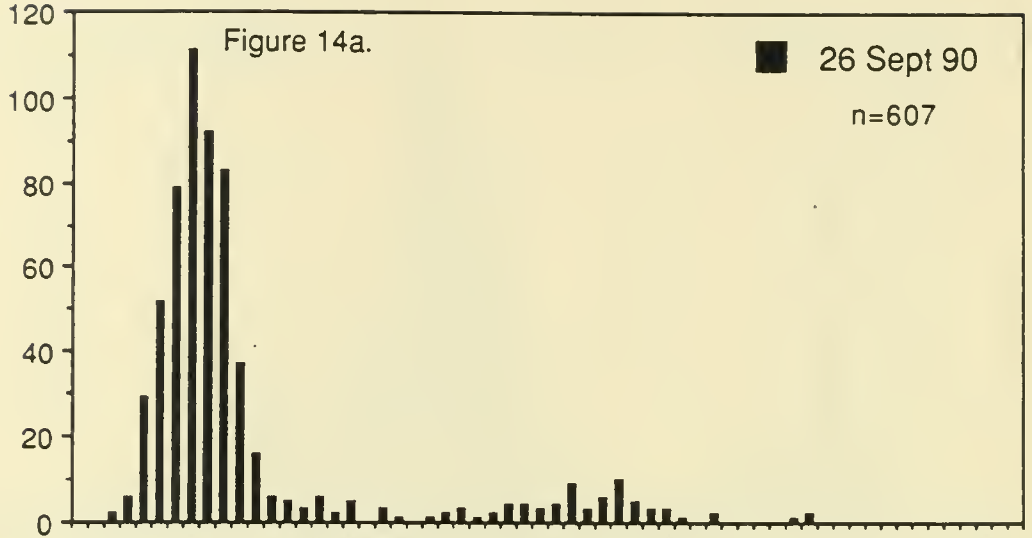


Figure 14a.

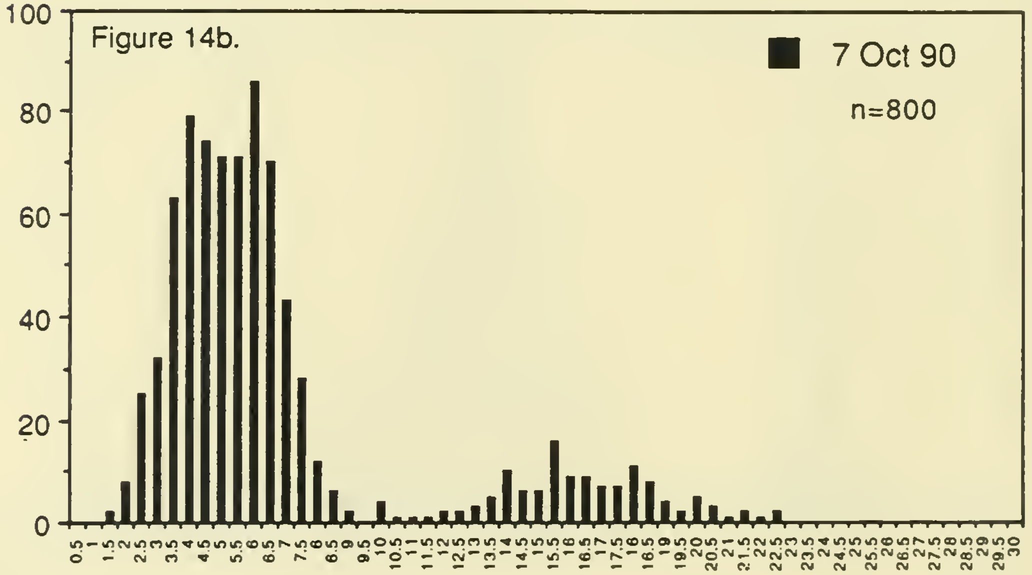


Figure 14b.

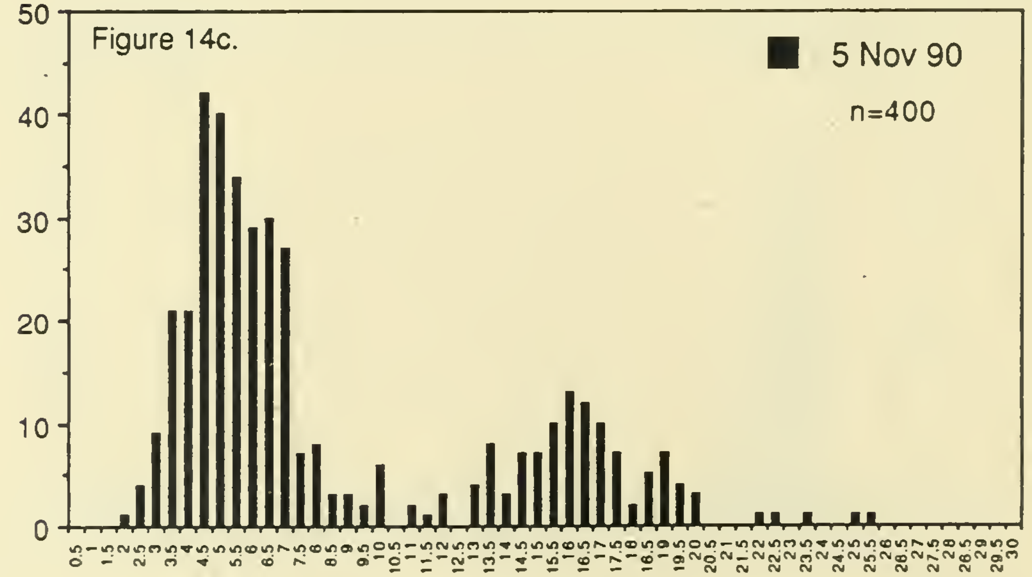


Figure 14c.

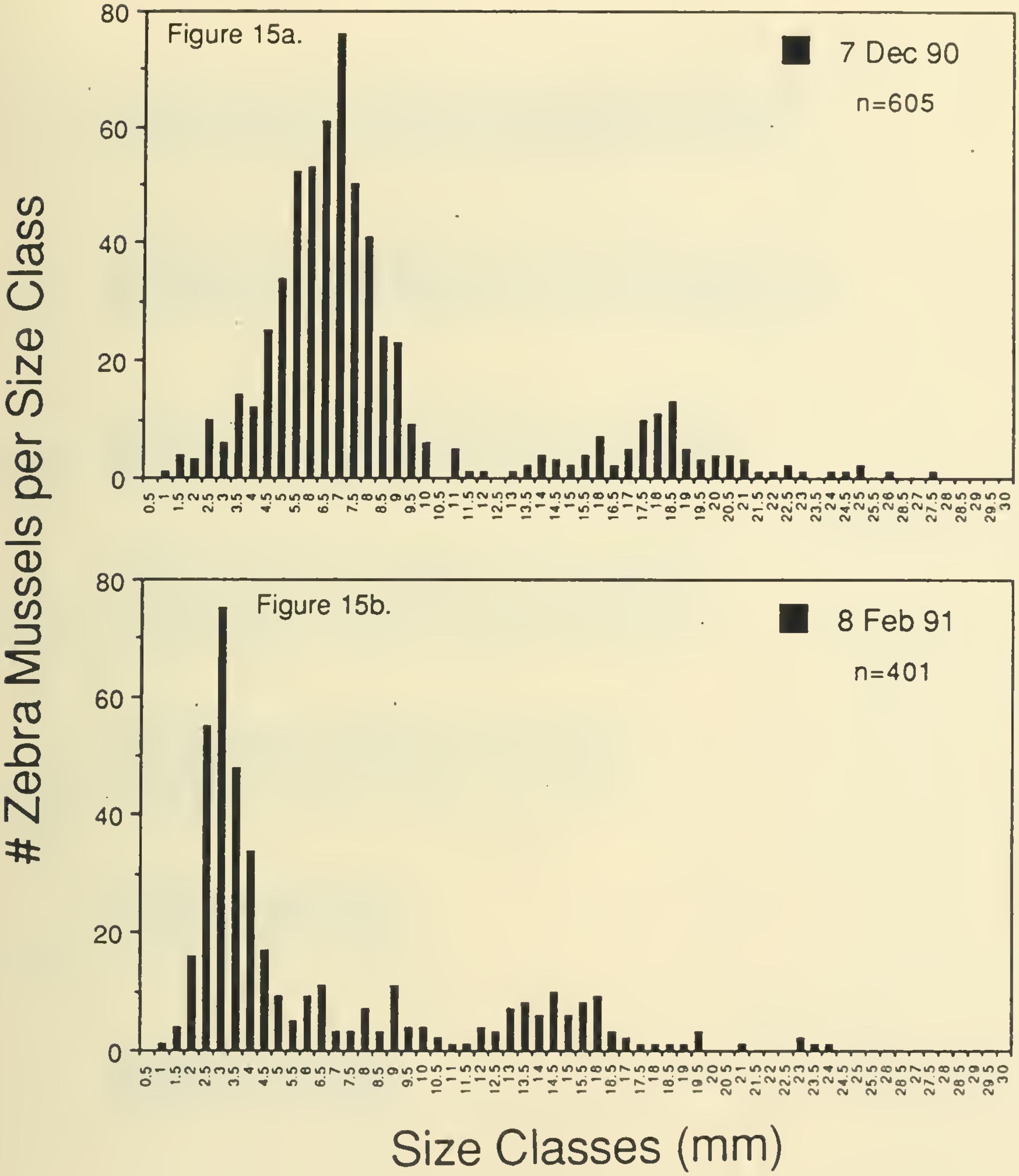


Figure 15a.

Figure 15b.

Figure 16. Growth Rates of the Dominant 1989 & 1990 Cohort in Lake St. Clair, at Puce.

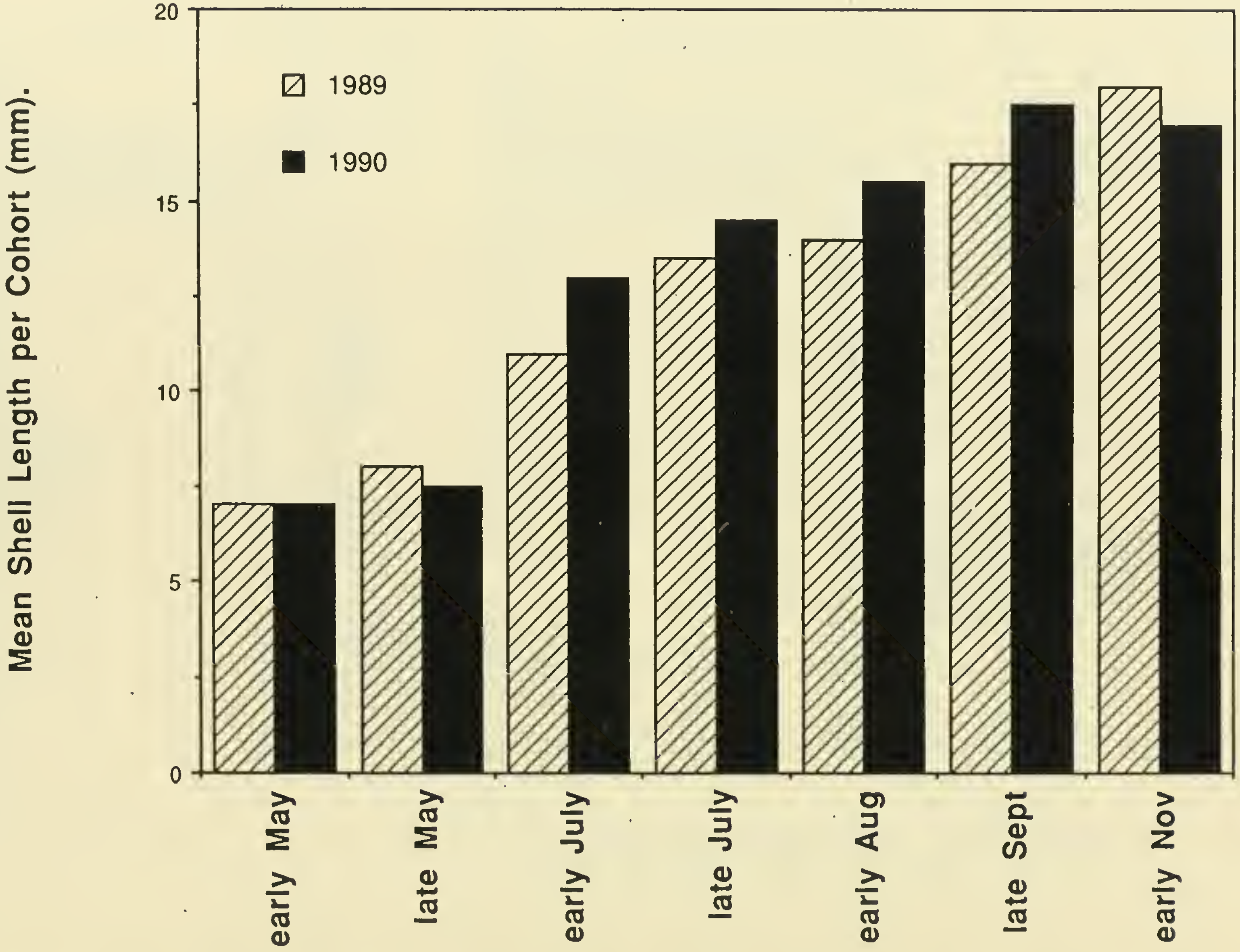
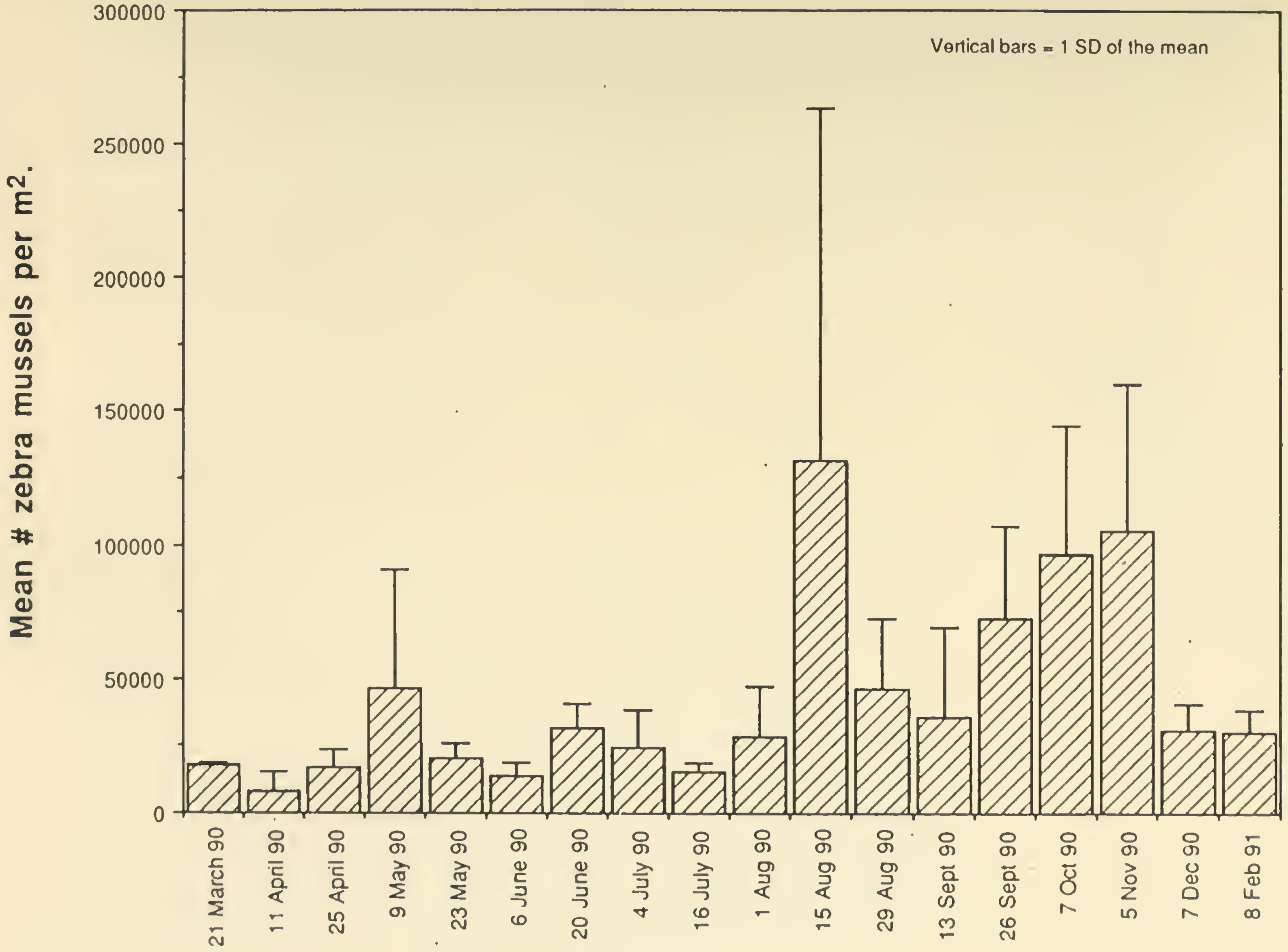


Figure 17. Seasonal variations in mean number of zebra mussels per m^2 of hard substrate from Lake St. Clair, at Puce.



IMPACTS OF THE EXOTIC ZEBRA MUSSEL, DREISSENA POLYMORPHA, (BIVALVIA: DREISSENIDAE) ON NATIVE UNIONIDAE IN LAKE ST. CLAIR

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SUMMARY

The exotic zebra mussel that has recently colonized the Great Lakes is hypothesized to have a severe impact on the native bivalves, especially of the family Unionidae. The impact of the zebra mussel on native Unionidae was examined in the south-western region of Lake St. Clair from 1989 to 1991. By 1990, 100% of the unionids at the Puce, Ontario site in Lake St. Clair were encrusted with zebra mussels. Heavy infestations of 1,000 zebra mussels or more per unionid are common in the study area. The mean number of zebra mussels found attached to living unionids at the Puce site was 152 in 1989 and 639 in 1990. The population of unionids in Lake St. Clair was surveyed in 1990 and 1991 to determine if colonization of unionids by zebra mussels is affecting the density and diversity of unionids. There appears to have been a decline in the unionid populations at the Puce site since the arrival of the zebra mussel. In a 1986 survey, Nalepa (1988), reported 7.8 unionids.m^{-2} in the offshore waters near Puce, Ontario. Our study found that the density of living unionids from the same study area had dropped to 1.74.m^{-2} in 1990 and to 0.06.m^{-2} in 1991. In 1990, eleven species were found alive but in 1991 only four species were found living. The majority (64%) of dead unionids collected in 1991 at the Puce, Ontario site had zebra mussels or byssal threads attached to their shells, suggesting that the zebra mussels are responsible for the decline in the unionid population. A second offshore site in Lake St. Clair, Grosse Pointe Farms, Michigan was sampled in 1991. The unionids at this site had only 15 zebra mussels.$unionid^{-1}$ in June but after the 1991 settlement the numbers of attached zebra mussels rose to around 700.$unionid^{-1}$. The density of unionids at the Grosse Pointe Farms site does not appear to have changed since the survey in 1986 by Nalepa (1988). Living unionids were found with massive colonies of zebra mussels on the posterior region resulting in occlusion of the unionid's siphonal region. The growth of zebra mussels between the unionid's valves prevents the total closure of the shell, apparently exposing the unionid to predation and/or parasitism. The weight of some zebra mussel

colonies is 3x that of the unionid they are attached to. This mass of zebra mussels appears to interfere with the unionid's locomotion and balance, causing the unionid to topple over onto its side. During sampling, many heavily-infested unionids have been found dead on their side. To determine if the zebra mussels are having an effect on the filtering activities of the unionids the clearance rates of infested and noninfested unionids are also being investigated. The growth rates and lipid stores of unionids in relation to zebra mussel densities are being investigated to further asses the impact of zebra mussels on the Unionidae.

INTRODUCTION

Dreissena polymorpha, the exotic zebra mussel, was first found in North America in 1988 in the south-western region of Lake St. Clair (Hebert et. al. 1989). *Dreissena* is referred to as a biofouler because it can attach itself to almost any hard substrate by secreting byssal threads. Zebra mussels will colonize most abiotic structures, such as rocks and intake pipes, as well as other living members of the benthic community such as large molluscs, particularly native bivalves of the family Unionidae. Zebra mussels will settle on any part of the unionid's valve that is exposed to the water column. This first layer of mussels forms the substrate for subsequent layers. Eventually, many layers of zebra mussels settle, resulting in a large mass attached to the posterior region of the unionid.

Dreissena polymorpha has also been found to colonize the native unionids in European Lakes. Only one study investigated the possibility of zebra mussels impacting the unionids. Lewandowski (1976) found that zebra mussels had no unfavourable effects on infested unionids. The infestation of unionids in Lake St. Clair appears to be more intense then the infestations in Europe and therefore any impact would be more evident. The average number of zebra mussels per unionid in the European study was 52 (Lewandowski 1976). This study found an average of 639 zebra mussels attached to the unionids from Lake St. Clair in 1990.

The objective of this study is to determine if the zebra mussels are having an impact on the native Unionidae in Lake St. Clair. The population of unionids in the south-western region of Lake St. Clair was surveyed in 1990 and 1991 to determine if colonization of unionids by zebra mussels is effecting the density and diversity of the unionid population. Laboratory experiments are presently being conducted to determine how the zebra mussels might be affecting the Unionidae.

MATERIALS AND METHODS

The south-western region of Lake St. Clair was chosen because the first North American sighting of *Dreissena polymorpha* was in this vicinity in 1988. Therefore, if there is an effect of the zebra mussels on the native unionids, this should be most evident in an area where *Dreissena* has been colonizing unionids for the longest period of time. In 1990 sampling was conducted in the offshore waters at the mouth of the Puce River near Puce, Ontario (Fig. 1). In 1991, sampling was conducted at both the Puce site and in the offshore waters of Grosse Pointe Farms, Michigan (Fig. 1). Puce was chosen as the main sampling site because of the large and diverse unionid population found there by Nalepa (1988) in the most recent survey of the unionid population in Lake St. Clair before the introduction of the zebra mussels. Nalepa (1988) reported that the unionid population in the area of Puce was 7.8 living unionids per square metre; this was the highest density in Lake St. Clair at that time. The second site, Grosse Pointe Farms was chosen because all unionids collected at the Puce site had attached zebra mussels, and a control population of unionids was needed. In 1991 the unionids at Grosse Pointe Farms were reported to have very few, if any, attached zebra mussels (Tom Nalepa, pers. comm.).

The density and diversity of the unionid population in Lake St. Clair were surveyed during 1990 and 1991 to assess the effect of the zebra mussel. All collections were done by hand using SCUBA and a 1 m^2 grid. Surveys were conducted once a month from June through September in 1990 and 1991. At each of 1, 2, 3 and 4 meters depth a 1 m^2 frame was haphazardly dropped on the substrate for a total of twenty replicates, and all living unionids and unionid shells within the frame were collected and identified to species using Clarke (1981). The identifications were then later compared with the collection of Unionidae from the Canadian Museum of Nature, Ottawa, Ontario for confirmation.

In order to determine the numbers of attached zebra mussels on unionids, *Proptera alata* was chosen as a representative species for detailed study in 1989 and 1990. The species has a large wing and a thick shell. *Proptea alata* was relatively abundant in 1990 at the Puce site and relatively easy to identify when encrusted with a large colony of *Dreissena*. Five *P. alata* were collected every sampling date from the 1-m depth, preserved in 70% ethanol, and brought back to the laboratory. In 1989, *P. alata* specimens were collected once a month from July to November. In 1990 *P. alata* specimens were collected once in March and April, every two weeks from May 29th until September 17th, then once in each of October and November.

For all the unionids collected in 1989 and 1990, the surface area of the valves projecting out of the substrate and exposed to the water column, the area that was covered by zebra mussels and the area submerged in the substrate, were all determined with aluminum foil. Each of the three areas were etched directly on the unionid's valve with an engraver. After all the zebra mussels were removed (see below for procedure) the unionid was wrapped in aluminum foil to the engraved lines for each of the areas. The weight of the aluminum foil for each area was then determined. The weights were converted to areas using the regression line of weight of foil on foil squares of known area.

All zebra mussels attached to the shells of the unionid were counted and the size frequency distribution was determined by measuring the lengths of a representative subsample of about 40-60 attached zebra mussels. This subsample of 50 zebra mussels was obtained by removing the attached mussels from an area approximately 2 cm^2 on the unionid's valve. The area included all layers, from the largest mussels, attached directly to the valve, to the newly settled young-of-the-year attached to the large mussels. The zebra mussels from each of the five unionids per sampling date were measured with the aid of a digitizing tablet (Summasketch II), to the nearest μm. The size frequency distribution for each collection was determined for approximately 250 mussels (about 50 mussels for each of 5 unionids) using SAS. The length, width, height and dry weight (50° C for two weeks) of all the unionids were measured. The average number of zebra mussels attached to each centimetre of exposed unionid valve was determined by dividing the total number of zebra mussels attached to the shell by the area of unionid that was exposed to the water column.

Initial sampling at all the depths of the Puce site in 1991 resulted in extremely low densities of living unionids being found. Therefore, in order to determine if the paucity of living unionids in the study area was due to patchiness or to a real decline in the population, a much larger area was intensively surveyed for living unionids The area surveyed extended 2.4 km on either side of the Puce site. Thirteen sites were sampled, each 0.4 km apart, for a total distance of 4.8 km. The sites were selected in zig-zag fashion extending out from either side of the Puce site (Fig. 2). At each of the thirteen sites the 1-m^2 frame was haphazardly dropped on the substrate and surveyed for living unionids. This procedure was repeated 30 times. For ten of 30 replicates in a subsample area of 0.5 m^2 all dead and living unionids within the frame were collected and the percentage of the shell that was covered in zebra mussels or byssal threads was determined. A similar survey, but not as intensive, was conducted at five stations surrounding the Grosse Pointe Farms, site to determine the number of unionids living in a unit area (1 m^2) and the percentage of shell covered in zebra mussels and/or byssal threads.

RESULTS

In 1990, the average densities of living unionids at the Puce site ranged from $1.74.m^{-2}$ (SE = 0.24) at the 4m depth to $0.28.m^{-2}$ at the 1 and 2 m depths (Table 1). *Elliptio dilatata*, *Fusconaia flava* and *Lampsilis radiata siliquoidea* were the most abundant of the 11 species found alive in 1990 (Table 2). In 1990 the ratio of dead to living unionids ranged from 0.12:1 to 0.37:1.

The density of living unionids declined over the summer of 1990. The June average density of 3.1 living $unionids.m^{-2}$ found at the 4 m depth declined to 0.55 $unionids.m^{-2}$ by September of that year (Fig. 4). Thirty-seven percent of the unionids collected at 4 m in 1990 were living, or a ratio of 1.74:4.66 living to dead was found. When sampling resumed in 1991 at the Puce site the average density of living unionids at 4 m had dropped to 0.06 $unionids.m^{-2}$ (SE = 0.0340) and $0.05.m^{-2}$ at 1 m (SE = 0.035) (Fig. 4). This was a significant decline from 1990 for 4 m ($p = 0.0005$) and for 1 m ($p = 0.006$). Of the 11 species found alive in 1990, only three were found alive in 1991; they were *Fusconaia flava*, *Lampsilis ventricosa*, and *Ligumia nasuta* (Table 2). All species showed a sharp decline from 1990 to 1991. Only seven of these declines were significant ($p = 0.05$) because the 1990 densities of those living species were already close to 0 living unionids m^{-2}. In 1990 a monthly sampling effort at all four depths resulted in an average of 4.9 (SE = 0.847) living species being found. In 1991 the same monthly sampling effort resulted in an average of 0.545 (SE = 0.157) living species found. This is a significant ($p = 0.0002$) drop in the number of living unionid species per unit effort found between 1990 to 1991. The ratio of living to dead unionids was 0.02:1 at 4 m and 0.01:1 at 1 m..

The number of zebra mussels does not appear to be limited by the surface area of the unionid. The average number of zebra mussels on a living unionid increased from 17 in 1988, to $152.unionid^{-1}$ (range 0-644) in 1989, to 639 zebra $mussels.unionid^{-1}$ (range 57-1991) in 1990 (Fig. 5). The average number of zebra mussels per cm^2 of exposed unionid valve increased from 3.2 in 1989 to 8.2 zebra mussels cm^{-2} in 1990. The numbers of zebra mussels per unionid of the 1991 population of zebra mussels was not determined because the zebra mussels had begun to die and fall off the unionids.

The 4.8 km intensive survey at the Puce site strongly suggested that the paucity of living unionids at this site was not due to patchiness but rather to a widespread decline in native bivalves. In 1990, 98 living unionids were found in 70, 1 m2 grids sampled at the 4 m depth but in 1991 when 390, 1 m^2 grids were sampled only five living unionids were found, the average density being 0.019 $unionids.m^{-2}$. 64% of the unionids and unionid shells had attached zebra mussels or byssal threads.

The 1991 results from Grosse Pointe Farms were very different from those at Puce in 1991.

The average density of living unionids at the Grosse Pointe Farms site ranged from 2.38.m^{-2} at 4 m to 0.025 living unionids.m^{-2} at 1 m (Table 3). Twelve species of unionids were found living at this site (Table 4). 23.75% of unionids at Grosse Pointe Farms were found living over all four depths. The numbers of zebra mussels attached to the Grosse Pointe Farms population of unionids, drastically increased over the summer of 1991 (Fig. 7). In June 1991, only 28 zebra mussels with an average length of 1.2 cm were found on a single unionid but by September 1991 there was an average of 715 per unionid.

The Grosse Pointe Farms intensive survey resulted in 2.4 living unionids.m^{-2} and only 20% of the shells of living and dead unionids being covered in zebra mussels or byssal threads.

DISCUSSION

Hebert et al. (1991) and Mackie (1991) predicted that zebra mussels could have a serious impact on the native bivalves. There have been suggestions that the density and the diversity of the unionid population in the lake has been reported to be declining since the 1980's. Nalepa (1988) found a significant decline in unionid abundances in 1986 at two of three sites that had been surveyed three years earlier by the University of Windsor. There was also a report of a large die-off of mussels in Lake St. Clair in the summer of 1986. Although the sharp and sudden decline in the numbers of living unionids since the introduction of the zebra mussel appears to be much more pronounced than in any previous reports of declines.

The most recent survey before the introduction of the zebra mussel was conducted in 1986 by Nalepa. All samples in this survey were collected using SCUBA and therefore the results are directly comparable with those of our study. Nalepa (1988) reported 7.8 unionids.m^{-2} at the 5 m depth in the area of Puce. We found 1.74 living unionids.m^{-2} at the 4 m depth in June of 1990. This density had decreased to 0.06 living unionids.m^{-2} by June of 1991. These results show a drop in the number of live unionid species from 11 in 1990 to 3 in 1991. The intensive 4.8 km survey at the Puce site also confirmed that there was a massive die off of unionids between 1990 and 1991. In 1990, 98 living unionids were found in 70, 1 m2 grids sampled at the 4 m depth but in 1991, a survey with five times as many grids, only 5 living unionids were found. This represents a drop in density of 99% from 1990 to 1991 at the Puce site.

The ratio of living to dead unionids at the 4 m depth did not decrease significantly from 1990 to 1991 even though the density of living unionids dropped significantly. This may be because unionids are no longer able to anchor themselves into the substrate with their foot when they die and are easily washed into shallower waters. The densities of dead unionids at the 1 m depth did increase significantly from $2.0.m^{-2}$ in 1990 to $4.72.m^{-2}$ in 1991. The empty unionid shells appear to be washing in to the nearshore waters (1 meter depth) from greater depths. Waterfront home owners in the area have reported a large increase in the number of clam shells washing up on their beaches in the past two years. Therefore, these data suggest that the apparent decrease in the ratio of living:dead unionids at 4 m does not reflect the drop in numbers of living unionids by an increase in the empty shells but the ratio of living:dead at the 1 m depth does.

Dreissena polymorpha was first discovered in the Puce area in Lake St. Clair off the Belle River, Ontario in 1988. Before the decline in unionids between 1990 and 1991, zebra mussels had been infesting unionids in the area for four years (Hebert et. al., 1989). The average number of zebra mussels attached to a unionid increased from 17 in 1988 (Hebert, 1989) to 639 in 1990. The sharp decline in the unionid population density from $7.8.m^{-2}$ in 1986 to 0.06 living $unionids.m^{-2}$ in 1991 strongly suggests that the exotic zebra mussel is having an impact on the native bivalves.

When zebra mussels colonize the shells of unionids, they may be having a number of effects on the unionid. Both the zebra mussels and the unionids are filter feeders and since the densities of zebra mussels are so high relative to the unionids (8.2 zebra mussels/cm^2 of exposed unionid valve and up to $200,000.m^{-2}$ on abiotic substrates) they are hypothesized to be stripping the water of nutrients required by the unionids, and therefore, starving the unionid (Mackie, 1991). The large colonies of zebra mussels attached to the posterior region of the unionid, where its siphons are located, may be altering the unionid's feeding currents when they feed. Zebra mussels will cover any exposed part of the unionid shell, including the extreme edge of the valve. When zebra mussels grow in the gape of the unionid shell, they prevent the total closure of the unionid's valves and leave it vulnerable to predation, parasitism and noxious waters. Zebra mussels attached to the edges of the unionid's valves may also result in shell deformities of the unionid which may eventualy lead it's death. Zebra mussels can also inhibit the unionid from opening its valves if the byssal threads span the gape of the valves, which would interfere with its movement by preventing foot extension and/or interfere with siphoning by preventing the extension of its siphons. The layering effect of the zebra mussels results in massive colonies attached to the posterior region of the unionid. Up to 1,991 zebra mussels were found attached to one living unionid. The colonies of zebra mussels attached to Lake St. Clair unionids have been reported to be three times the weight of the unionid itself (Hebert, 1991). This mass of zebra mussels can cause the unionid to become unbalanced and topple over onto its side and, if the unionid is unable to right itself, it may die in that position. During sampling, many dead unionids have been found on their side with large colonies of zebra mussels attach to their shells. Any combination of the above effects may explain the significant decline in the unionid population at the Puce sampling site since the introduction of the zebra mussel.

The zebra mussel population found at Grosse Pointe Farms in 1991 was much smaller than the population at Puce in 1990. The average number of zebra mussels attached to a living unionid was 8.7 (SE = 2.10) at Grosse Pointe Farms in June of 1991. This was significantly less than the average of 639 attached to unionids at Puce in September of 1990. *Dreissena* did not appear to have had a noticeable impact on the unionid densities and diversity as of September 1990 since Nalepa (1988) reported 2.0 living unionids.m^{-2} at the same site in 1986; our study found 2.4 living unionids.m^{-2} for 1991. In September 1991 the average number of zebra mussels at Grosse Point Farms had increased to 714 per unionid. The majority of the zebra mussels were young-of-the-year. As the newly-settled zebra mussels grow and reproduce there is a strong possibility of negative impact on the Grosse Pointe Farms unionid population. If in fact the infestations of zebra mussels have caused the unionid population to decline at Puce, then the Grosse Pointe Farms population will most likely follow the Puce population and begin to decline in the next two years.

Zebra mussels have been found to colonize all species of unionids in the study area. The shape of the unionid appears to be a factor in determining the degree of impact of the zebra mussel. *Leptodea fragilis* is an extremely thin-shelled, large-winged unionid. An accumulation of zebra mussels on the wing area appears to alter the unionid's balance, causing the clam to topple over. Nalepa (1988) reported that *L. fragilis* was the second most-common species found in Lake St. Clair in 1986. This study found 0.017 *Leptodea fragilis* per m^2 at the Puce site in 1990. *L. fragilis* was only the nineth-most abundant of the eleven species found in 1990 and by 1991 there was no living *L. fragilis* found. The thin-shelled unionids appear to have been affected by the zebra mussel infestation more than the thicker-shelled species such as *Fusconaia flava*, *Lampsilis ventricosa*, *Elliptio dilatata*, and *Ligumia recta*, which were still relatively abundant in 1990. These species are all somewhat rounded and thick-shelled. The only three species of unionids found living in 1991 at Puce were ones that are rounded and heavy-shelled, probably not as susceptible to tipping as the thin-shelled and alate species, such as *Leptodea fragilis*.

According to Nalepa (1988) the area around Puce had the highest density of unionids in Lake St. Clair in 1986. In the three years since the discovery of *Dreissena polymorpha* the unionid population has been almost eliminated. Perhaps in areas with lower densities of unionids, the population may decline even faster and some species will become locally extinct.

ACKNOWLEDGEMENTS

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Table 1. Density of Living and Dead Unionids at Puce, Ont. in 1990 and 1991.

Depth (Meters)	Density Living ($\#/m^2$)			Density Dead ($\#/m^2$)		
	1990	1991	Significant Decline	1990	1991	Significant Increase
1	0.275	0.05	p=0.006	2.0	4.72	p=0.0078
2	0.28	0.00	p=0.0001	2.38	4.28	p=0.0315
3	1.00	0.00	p=0.00005	4.66	3.98	p=0.419
4	1.74	0.06	p=0.00005	4.66	3.77	p=0.246

Table 2. Density of Living Unionids by Species at Puce, Ont. in 1990 and 1991, Averaged Over Four Depths.

Unionid Species Found	Density/m^2 1990	Density/m^2 1991
Amblema plicata (Say)	0.023	0*
Elliptio dilatata (Rafinesque)	0.270	0**
Fusconaia flava (Rafinesque)	0.227	0.0165**
Lampsilis ventricosa (Rafinesque)	0.081	0.004**
Lampsilis radiata siliquoidea (Barnes)	0.089	0**
Leptodea fragilis (Rafinesque)	0.017	0
Ligumia nasuta (Say)	0.010	0.004
Obvaria reflexa (Rafinesque)	0.013	0
Pleurabema cordatum f. coccineum (Rafinesque)	0.037	0**
Ptychobranchus fasciolaris (Rafinesque)	0.019	0**
Proptera alata (Say)	0.047	0**
Total Number of Species Living	11	3

* "0" Density indicates that only empty shells of these species were found.

** Indicates a significant difference in the density of this species from 1990 to 1991 (p=0.05).

Table 3. Density of Unionids at Grosse Pointe Farms, MI at each of 4, 3, 2 and 1 meters depth.

Depth (Meters)	Density Alive #/m^2	Density Dead #/m^2	Percent Living Alive/Dead
4	2.377 (SE 0.228)	7.60 (SE 1.201)	31 %
3	1.188 (SE 0.158)	7.96 (SE 1.668)	15 %
2	0.838 (SE 0.202)	2.275 (SE 0.522)	37 %
1	0.025 (SE 0.025)	0.188 (SE 0.064)	12 %

Table 4. Density of Living Unionids by Species at Grosse Pointe Farms, MI in 1991, Averaged Over Four Depths.

Unionid Species Found	Density Living/m^2
Anodonta grandis grandis (Say)	0.066
Amblema plicata (Say)	0.030
Elliptio dilatata (Rafinesque)	0.110
Fusconaia flava (Rafinesque)	0.087
Lampsilis ventricosa (Rafinesque)	0.127
Lampsilis radiata siliquoidea (Barnes)	0.120
Leptodea fragilis (Rafinesque)	0.127
Ligumia nasuta (Say)	0.034
Obvaria reflexa (Rafinesque)	0.003
Ptychobranchus fasciolaris (Rafinesque)	0.033
Proptera alata (Say)	0.318
Truncilla truncata (Rafininesque)	0.130
Total Number of Species Living	**12**

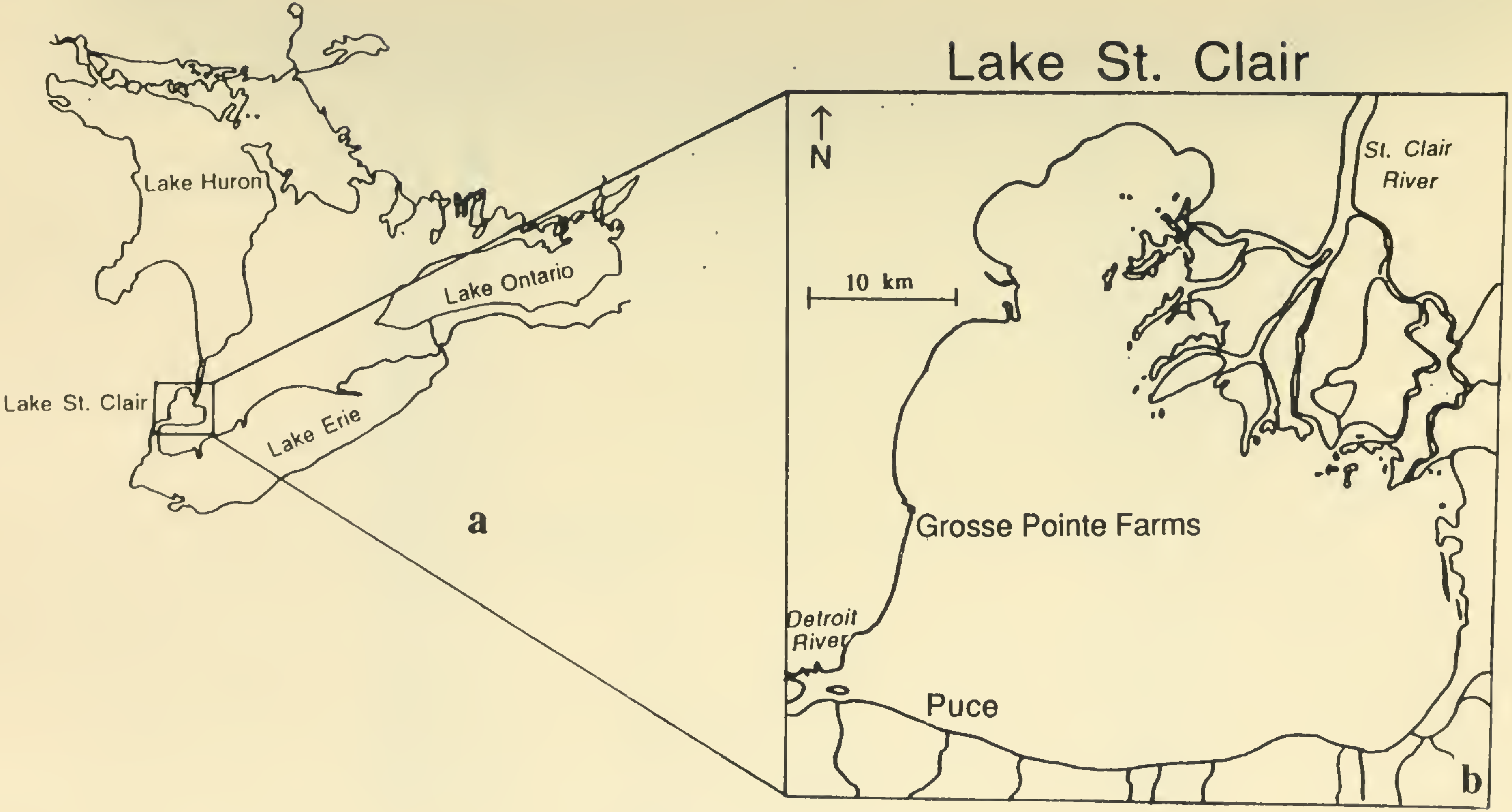

Figure 1. Map of Lake St. Clair showing sampling sites. Puce, Ontario was sampled from 1989 to 1991. Grosse Pointe Farms, Michigan was sampled in 1991 only.

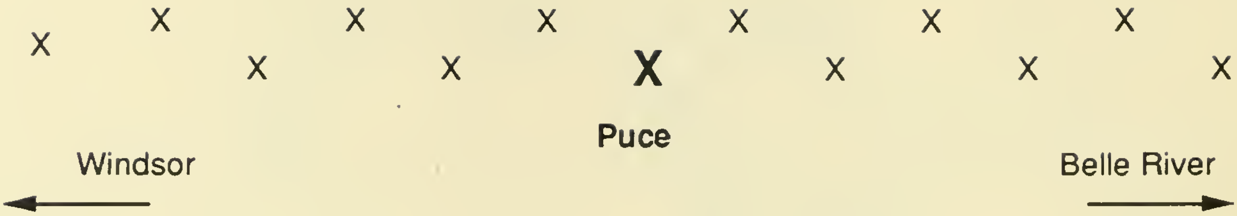

Figure 2. Intensive Survey For Living Unionids at Puce in 1991

-Thirteen sites 0.4 km apart were sampled.
-The total distance surveyed was approximately 4.8 km.

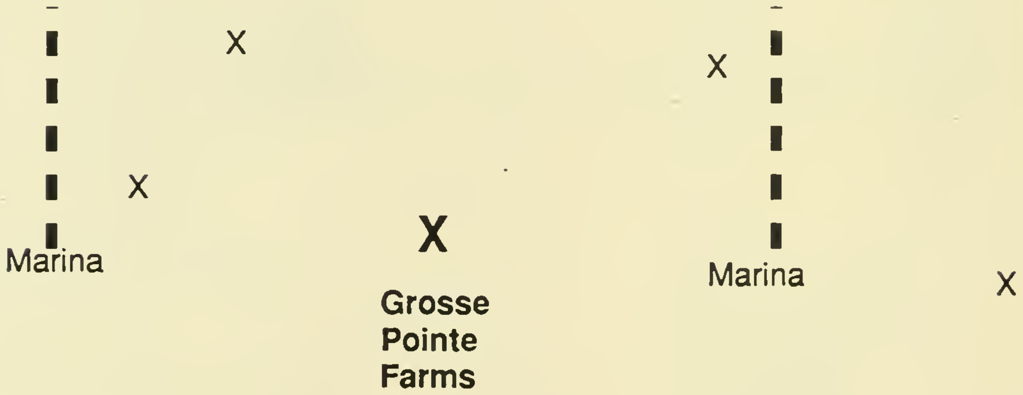

Figure 3. Intensive Survey For Living Unionids at Grosse Pt. Farms in 1991

-The five sites were various distances apart due to the marinas.
-The total distance surveyed was approximately 3.5 km.

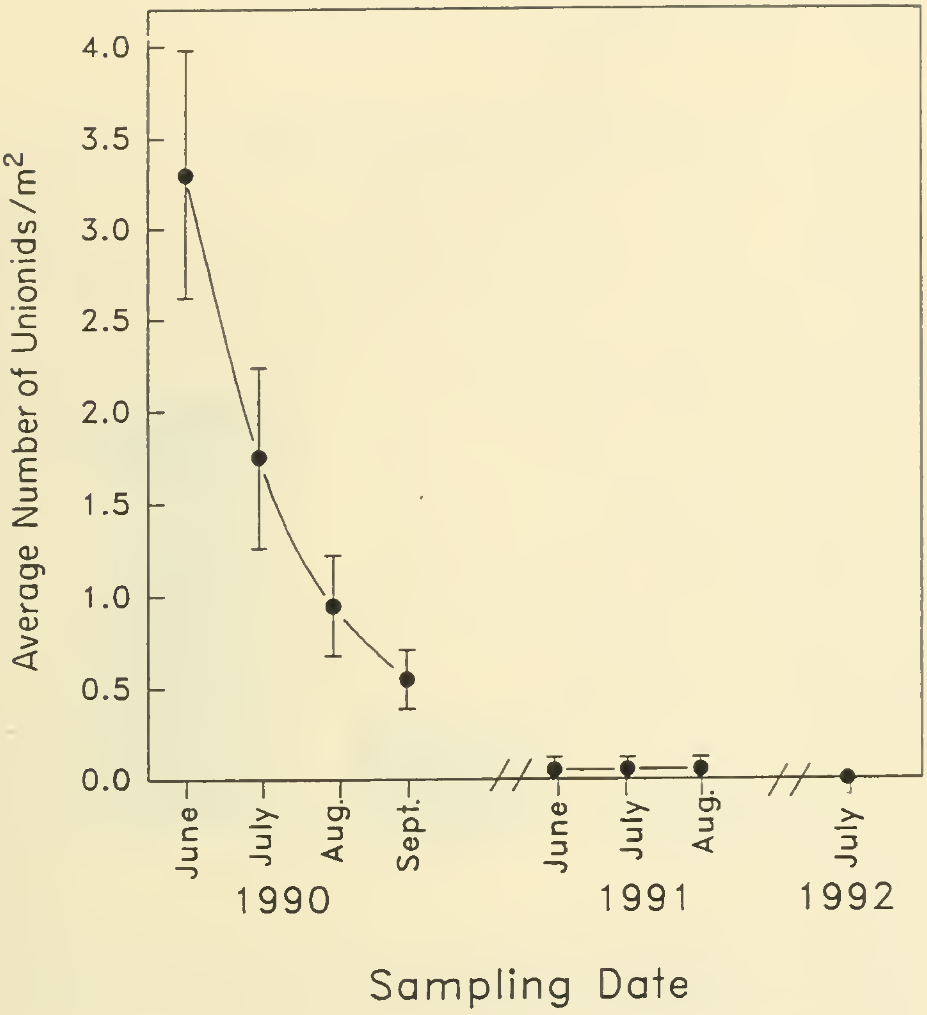

Figure 4. Density of living unionids from the 4-m depth at Puce, Ontario, June 1990 to July 1992. Each point represents the mean from 20 replicate samples. Vertical bars = 1 standard error about the mean.

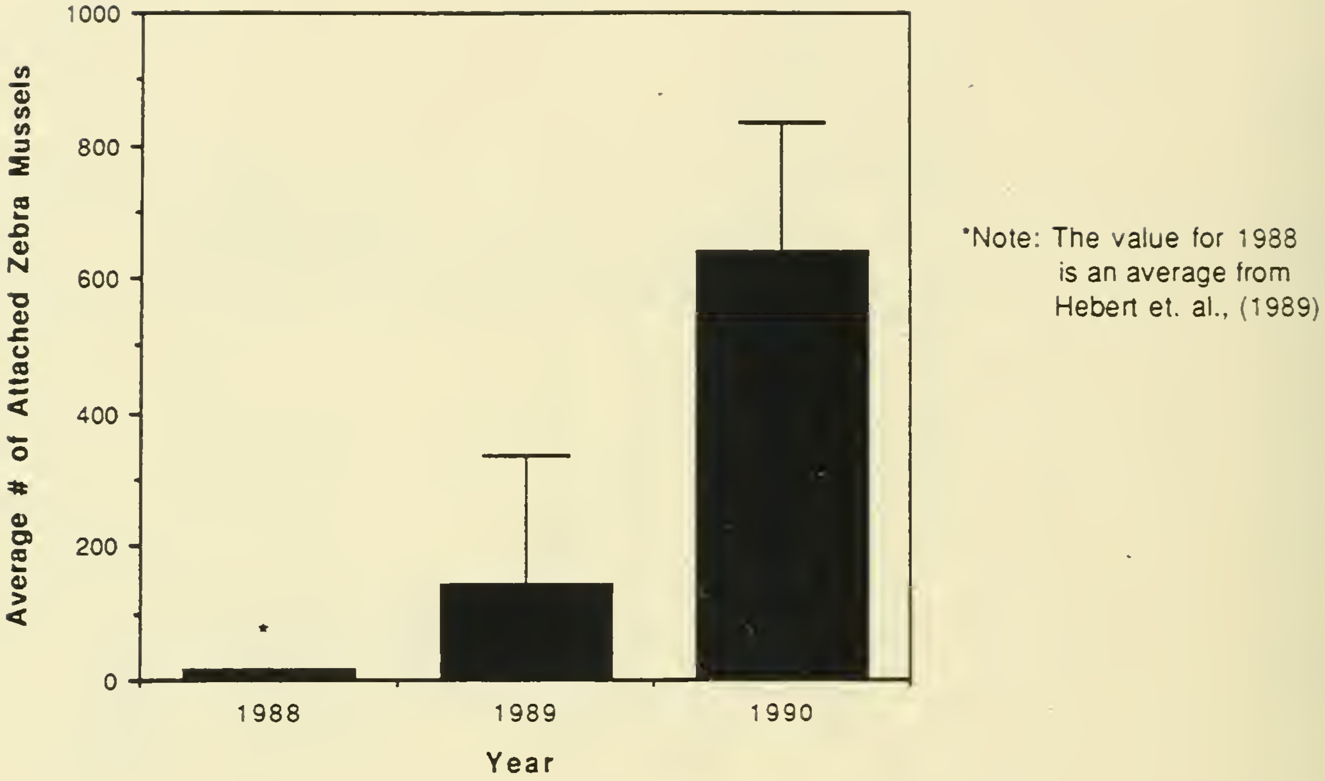

Figure 5. Average Number of Zebra Mussels Attached to Unionids at Puce, Ont. 1988-1990.

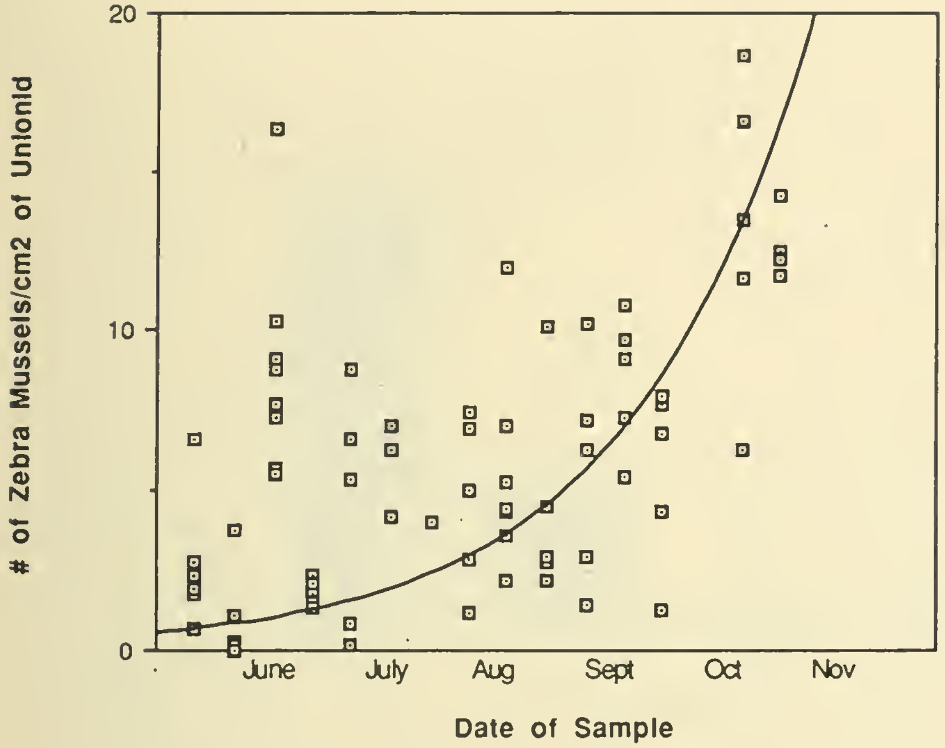

Figure 6. Number of Zebra Mussels per cm2 of Exposed Unionid Valve, at Puce June 1990 - Nov. 1990.

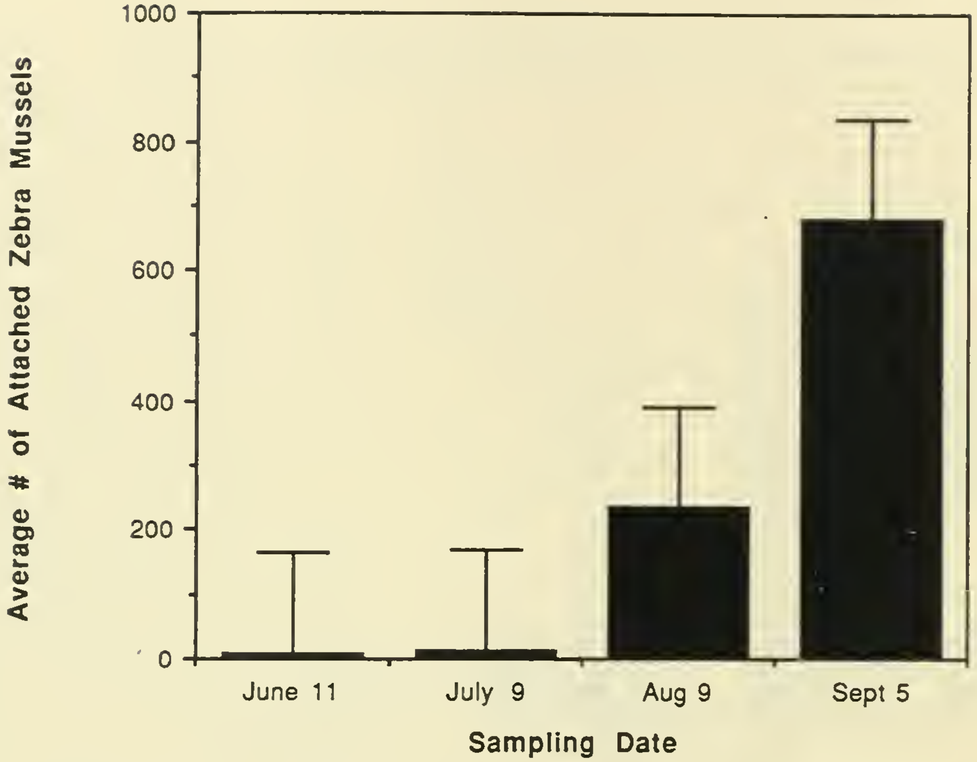

Figure 7. Average Number of Zebra Mussels Attached to Unionids at Grosse Pt. Farms, MI June-Sept. 1991.

THE EFFECT OF EPIZOIC *DREISSENA POLYMORPHA* ON THE FILTERING ACTIVITIES OF THE HOST UNIONID, *PROPTERA ALATA*

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SUMMARY

Massive infestations of up to 2000 zebra mussels per unionid shell usually results in total occlusion of the siphon openings of the host unionid. Laboratory studies were performed to determine if the attached mussels interfere with the food aquisition of the unionids by comparing clearance rates of infested and uninfested native mussels of the species, *Proptera alata*. Using four different algal (*Chlamydomonas reinharddii*) concentrations (5,000, 10,000, 30,000, and 50,000 cells/mL) and four levels of zebra mussel infestations, the studies showed that the position of the attached mussels on the unionid shell is an important factor in determining if zebra mussels interfere with the filtration activities of the unionid. Zebra mussels attached to the siphonal area block the siphons of the unionid and physically interfere with its siphoning activities.

INTRODUCTION

Dreissena polymorpha, the recently introduced zebra mussel, is an epifaunal bivalve that will colonize most hard substrates by secreting adhesive byssal threads. Zebra mussels are well known as nuisance biofoulers because they colonize abiotic substrates such as rocks and the intake pipes of industry. High densities of *Dreissena* also have negative ecological impacts when they attach to other living members of the benthic community, including the native bivalves of the family Unionidae.

Freshwater Unionidae are infaunal bivalves that burrow into the mud, anchoring the anterior portion of their shell into the substrate with their foot. The posterior region of the shell remains exposed to the water column and therefore is susceptible to colonization by zebra mussels. As with any zebra mussel colony, the oldest mussels are attached directly to the substrate, in this case the shell of the host unionid. Younger mussels continue to settle on top of the colony, resulting in a large mass of zebra mussels on the posterior region of the unionid. The number of zebra mussels attached to a single North American unionid has been reported to be as high as 10,000 (Schloesser and Kovalak, 1991 and Hebert et.al., 1991) and the weight of the *Dreissena* colony can be up to 17 times the weight of the unionid itself (Lewandowski, 1976). We have found a maximum of 1,991 zebra mussels attached to a single specimen of *Proptera alata* collected from Lake St. Clair in 1990. Zebra mussels appear to be nonselective in the unionids they colonize. All species of Unionidae in the study area of Lake St. Clair (see Appendix I for map) have been found with attached zebra mussels. Hebert (1991) states that in soft sediment lakes, unionids may be the only available hard substrate. Biryukov et. al. (1964) and Lewandowski (1976) found that *Dreissena* prefer to colonize the shells of living unionids over empty shells and rocks.

The respiratory and feeding appendages of a unionid, the siphons, are housed in the posterior region of the unionid that is colonized by zebra mussels (Fig. 1). Unionids with large colonies of attached zebra mussels often have their siphons obstructed or completely blocked by zebra mussels. When the zebra mussels surround the unionid's siphon, they may create currents which interfere with the siphoning activities of the unionid. The zebra mussels attached to the siphonal region of the host unionid may also be physically interfering with the food acquisition and respiration of the unionid, thereby having a negative effect on the unionid. Both *Dreissena* and the Unionidae are suspension feeding bivalves. Suspension feeding involves clearing large volumes of the surrounding water to obtain food for maintenance, growth and reproduction (Jorgensen, 1975). When the extremely high densities of zebra mussels (200,000/m^2, Mackie, 1991) filter feed they may be stripping the water column of nutrients required by the native bivalves and thereby starving the unionids. There is also a possibility that there may be competition for food resources between these two groups.

Prior to the introduction of zebra mussels into North America, the most in-depth European study on the effect of zebra mussels on unionids (Lewandowski, 1976) reported that an intensive overgrowth of zebra mussels appeared not to have any unfavourable effect on the mean body weight of unionids. Lewandowski (1976) did, however, find that zebra mussels had an inhibitory effect on the growth rate of younger unionids, up to four years old. Lewandowski (1976) did not experimentally investigate the possibility that zebra mussels physically interfere with the filtering activities of unionids, but he did report some qualitative observations. He observed strong deformities and a general change in shape of the posterior (siphon) region of the unionid's shell when a large number of zebra mussels were attached. Lewandowski, (1976) also suggested that a large number of settled *Dreissena* may mechanically impede the inflow of water into the unionid's overgrown siphon, and suggested that the water flowing into a siphon of the unionid may be impoverished in nutrient substances. There have been recent reports of *Dreissena polymorpha* having negative effects on populations of native unionids (Garton, 1992; Mastellar, 1992; Schloesser and Kovalak, 1991 and Gillis and Mackie, 1991). To date in North America there have been no investigations into the actual mechanism by which zebra mussels affect unionids, only suggestions (Mackie, 1991 ; Hebert et. al., 1991; Schloesser and Kovalak, 1991) that zebra mussels may interfere with the normal functioning of the unionid's siphons. Hebert et. al., (1991) suggested that *D. polymorpha* may be impacting the unionids in two ways: they may act as exploitative competitors or they may be interference competitors.

The objective of this study is to experimentally determine if zebra mussels physically interfere with the siphoning activities of unionids when they overgrow the unionid's shell and occlude the siphonal area. Only the possibility of interference competition was investigated and no attempt was made to determine if Unionidae and *Dreissena polymorpha* are involved in exploitative competition.

REVIEW OF FILTRATION AND METHODOLOGY

To determine if zebra mussels interfere with the filtering activities of unionids, the filtration rates of infested unionids and uninfested unionids were compared. We assumed that zebra mussels colonizing the siphonal area of a unionid physically interfere with the food acquisition of the unionid and when the attached zebra mussels are removed the physical interference is also removed. The experiment was divided into two parts: Part I involved determination of the clearance rate of unionids with the zebra mussels still attached (referred to here as "Attached"). Part II of the experiment involved determination of the clearance rate of unionids when zebra mussels were removed but present in the same experimental aquarium (referred to here as "Detached"). The difference in filtration rate between

Part II and Part I (i.e. Filtration rate Detached - Filtration rate Attached) provides an estimate for physical interference.

Filtration rate is defined as the volume of water filtered completely free of particles per unit time (Winters, 1978). Historically, there have been two approaches to measuring the filtration rate of bivalves; the indirect method and the direct method. The direct method involves manipulation of the siphons to separate the inhalant siphon from the exhalant siphon and to intercept and measure the feeding current of the animal (Coughlan 1969). The indirect method involves measuring the amount of suspended algae that is removed from suspension per unit time, or the clearance rate (Davids 1964). Clearance rate is then used in a formula developed by Coughlan (1969) to calculate filtration rate.

Coughlan (1969) states that four assumptions must be made before one can calculate filtration rate from clearance rate.

1. The reduction in the concentration of particles is due to the filtration of the animals and gravitational settling.
2. The animal's pumping rate is constant.
3. Particle retention is 100% efficient.
4. The test suspension is at all times homogeneous.

Given these conditions, the filtration rate calculated would be the volume of water actually pumped by the animal in a given period of time (Coughlan, 1969).

These assumptions were made in the present experiment, with the following provisals: 1. A control experiment including water, substrate and algae was conducted to account for any change in the concentration of algae that was not caused by the experimental animals. 2. It is unknown if the pumping rate was constant during the experiments. Sprung and Rose, (1988) found that pumping was not continuous even though the shells of the experimental bivalves were open at all times. If the pumping rate of either zebra mussels or unionids varied during the experiments, one would expect that any changes would be random or directly correlated to the experimental parameters. 3. Sprung and Rose (1988) found that zebra mussels show maximum retention of 5-μm diameter particles and will ingest most particles ranging in size from 0.7 μm to 35 μm. Jorgensen et. al. (1984) reported that unionids will retain particles with a diameter of 4 μm. The cells of *Chlamydomonas reinhardii* used in this experiment were within the acceptable size range for both groups of animals (see Methods for algal size). 4. We chose not to recirculate the aquaria water during the experiments in order to avoid resuspending the faeces and pseudofaeces produced by the experimental animals. Sprung and Rose (1988) and Walz (1978a) found that zebra mussel faeces disintegrate very quickly and cause a sudden increase in particle numbers. Also Davids (1964) stated that circulating the water to keep experimental particles in suspension would stir up the pseudofaeces.

The indirect method of determining filtration rates was chosen because it was difficult to manipulate the siphons of all the zebra mussels attached to a unionid. Also manipulations of experimental animals often result in unnatural results (Kryger and Riisgård, 1988). Schulte (1975) chose the indirect method because of the relatively low degree of disturbance of the filtering mussels during the experiments and because no prior preparation of the specimens was necessary. Schulte (1975) also stated that the indirect method allowed him to use clumps of fifty *Mytilus* in one experiment, as was the case in this study. Morton (1971) used the indirect method of measuring the clearance rate when determining the filtration rate of *Dreissena* because he felt that zebra mussels were too small to manipulate.

There are also problems associated with the indirect method of determination of filtration rate. Winter (1978) states that the main problem with the indirect method is the continuously changing food concentration in the tank. Davids (1964) further details the advantages and disadvantages of both of these methods. Many authors who used one method of determining filtration rate disagree with the other, but Kryger and Riisgård (1988) used both the indirect and direct method for zebra mussels and found the results agreed closely.

Unicellular algae was used as experimental particles as opposed to polystyrene beads and inorganic particles because some animals reject these unnatural food particles. DeMott (1986) found that some suspension-feeding animals select their food particles by taste and would only ingest polystyrene beads that were flavoured with lysed algae. In the past (Walz 1978a; Morton, 1971 and Jorgensen, 1960) used inorganic suspensions, such as colloidal graphite, aquadag, and prodag. Kryger and Riisgård (1988) stated that the use of these particles results in unnaturally low filtration rates because they may chemically irritate the suspension feeder due to the extremely high concentrations needed to be detected photometrically. Newell and Jordan (1983) found that oysters preferentially ingested organic particles and rejected the inorganic particles through the production of pseudofaeces. Newell and Jordan (1983) suggested that the nutritious organic component of the seston was actively selected and particles that did not meet the sensory criteria may have been allowed to move to the resorting or rejection ridges. As with any unnatural foods, there can be problems associated with the single species algal suspension used in this experiment. Rice and Smith (1958) reported higher filtration rates in suspensions of mixed diatoms than in uni-algal suspensions. Palmer (1980) stated that single species algal suspensions used in laboratory experiments can only be extended with caution to field conditions where animals are filtering a mixed diet of naturally occurring algae.

Animals were acclimated to the experimental concentration of algae 24 hours before the start of Part I of the experiment. Davids (1964) reported that the pumping rate of *Mytilus* depended on what was offered to the animal before the experiment. He found that if a test suspension is followed by one of a lower concentration then the pumping will increase and if a test suspension is followed by a more concentrated one then as a rule, pumping rate will decrease. Ten Winkle and Davids (1982) fed algae to *Dreissena* 24 hours before the experiment because they felt that the selection mechanism may operate abnormally in starved individuals. Ten Winkle and Davids (1982) also stated that the selection mechanism did not function at all in animals that had been starved for 16 hours

MATERIALS AND METHODS

Unionidae

Unionids of the species *Proptera alata* encrusted with *Dreissena polymorpha* were collected from the south-western region of Lake St. Clair in November 1991. All *P. alata* were collected on the same day from a depth of 4 metres offshore at Grosse Pointe Farms, Michigan (see Appendix I for map). *Proptera alata* were held in well-aerated water in 120-L glass aquaria at room temperature. Each aquarium contained 8 to 10 cm of beach sand in the bottom to simulate the natural environment and allow the unionids to bury themselves in the substrate. Kryger and Riisgård (1988) found that unionids lying on their side on top of the sediment always had a reduced filtration rate. It was necessary in this experiment that the unionids remain upright in their natural position to prevent the attached zebra mussels from being smothered.

The holding aquaria were cleaned and the water (1:1 well water:deionized water) was replaced twice weekly. Each aquarium contained 8 to 12 unionids, depending on the size of the unionids. The unionids were fed freeze-dried *Chlorella* sp. tablets reconstituted with deionized water throughout the period of holding.

Algae Cultures

Chlamydomonas reinhardii, University of Toronto Culture Collection strain #84 was cultured in 100% Bolds Basal Medium (BBM) as a food source for clearance rate experiments (see Appendix II for medium recipe). This species of algae was chosen because it is a flagellate, unicellular green alga and its mobility would tend to minimize the amount of algae that settled out of suspension during the experimentation. The culture consisted of eight, 20-L clear polycarbonate carboys. When the concentration of algal cells reached 1.5 million cells/mL, the culture was diluted by one half with BBM. The cultured required dilution every five to six days. All transfers of algae and additions of medium were conducted aseptically over a gas flame after swabbing all exposed surfaces with 100% ethanol since a laminar flow fume hood was not available. The culture was kept at room temperature (20°C) and surrounded by 'Gro and Sho' florescent lighting 24 hours a day.

All glassware used in the preparation of medium was washed with 5% hydrochloric acid. The individual chemical ingredients of the medium were frozen in proportions needed to prepare 20 L of medium in Fisher Brand 'Whorl Packs'. The medium was prepared using the required chemical ingredients and distilled-deionized water. The pH of the medium was adjusted to 6.86 using potassium hydroxide and hydrochloric acid. The completed medium was autoclaved for 20 minutes and allowed to cool to room temperature before adding to the cultures.

Particle Counts

All particles were counted using a Coulter Counter Model Z_m fitted with a 100 μm aperature tube. All samples were diluted with 'Isoton II', a 1% saline solution used to dilute and increase conductivity of the samples. The pure alga cultures were diluted 100:1 and the samples of experimental aquaria water were diluted 3:1 with the saline solution. Three counts were taken from each accuvette. A count consisted of determining the number of particles in 500 μL of the diluted sample. The size range of particles to be counted by the Coulter Counter was determined by measuring the cell dimensions of the elongate *Chlamydomonas reinhardii*. The width of the unicellular algae ranged from 3.35 to 5.5 μm and the length of the cell ranged from 6.6 to 8.8 μm. Algal cells undergoing cell division were more than 2x wider than a single cell. Since the angle at which the particles entered the Coulter Counter aperture could not be controlled, the Coulter Counter was set to count particles from 3 to 14 μm in order to catch all possible rotations of the algal cells. Algal cell counts as determined by the Coulter Counter were confirmed optically by a haemocytometer. After each experimental count, a blank of the saline solution was counted in order to clean the aperture tube. Counts were determined on the 'coincidence' correction setting. This feature statistically adjusts for the possibility of more than one particle entering the aperture at a given time according to the concentration of the sample.

Experimental Variables

The clearance rates of the experimental unionids and zebra mussels were determined at four algal concentrations; 5,000 cells/mL, 10,000 cells/mL, 30,000 cells/mL and 50,000 cell/mL. These concentrations were chosen to bracket the zebra mussel incipient limiting level of 16,000 cells/mL as determined by Sprung and Rose (1988). The sequence of experimental concentrations was randomized over time to prevent a bias that would occur if there were a decline in the health of the unionids over the course of the experiment (see Appendix IV for sequence).

The sizes of unionids used in the experiments ranged from 75.8 mm to 137 mm in shell length. Unionids with shell lengths less than 95 mm were labelled as 'small'; unionids with shell lengths between

95.1 and 110 mm were labelled as 'medium'; and unionids over 110.1 mm in length were labelled as 'large'. The clearance rates of three unionids from each of the small and medium size classes and two unionids from the large size class were determined at each of the four experimental concentrations of algae. When possible, one unionid from each of the three size classes was used in a daily set of experiments at a given concentration.

Experimental Design Checks

To determine the duration of the experiment that would yield a detectable drop in the concentration of particles, a trial experiment was conducted at 15,000 cells/mL which was close to the incipient limiting level for zebra mussels (Sprung & Rose 1988). Readings were taken at half hour intervals up to two and one half hours and the particle concentration of the aquaria water at each time was determined. After the time periods of thirty minutes and one hour there was no detectable difference in the number of particles from the control experiments (see Appendix V for Figure). All subsequent times showed a decline in the particle concentration. An experimental duration of one and one half hours was therefore chosen.

To determine the amount of water needed in the experimental aquaria, the filtration rate of a large colony of *Dreissena* was determined. All the zebra mussels were measured to the nearest micrometre with the aid of a 'Summasketch II' digitizing tablet. These lengths were converted to dry weights and the weights were then used to estimate filtration rates using equations proposed by Kryger and Riisgård (1988). These values were then used to determine the filtration rate of the host unionid and the entire population of attached zebra mussels (see Appendix VI for calculations). The filtration rate of a 120.1 mm long unionid with a population of 468 attached zebra mussels was calculated to be 12.6 L/hr for the zebra mussels and 2.6 L/hr for the unionid. This size of unionid would be classified as 'large' and the attached zebra mussel population contained 150 more zebra mussels than the largest population used in the experiments. Also, the Kryger and Riisgård (1988) equations assume that all zebra mussels are filtering during the experiment. Consequently, 15.2 L/hour is a much larger volume of water than the experimental unionids and attached zebra mussels would be filtering during an experiment. Therefore, aquaria that hold 16 L of water would be an adequate size for 1.5-h clearance rate experiments.

Sixteen, 20-L aquaria were used in the experiment. Beach sand that was used as substrate for the unionids covered the bottom of the aquaria, filling a space equivalent to four litres of water. The remainder of the aquarium was filled with deionized water. All tanks were equipped with a tubing and stopper system to allow for easy drainage and cleaning without the removal of the sand. To ensure the removal of all algae, faeces and pseudofaeces, the aquaria were thoroughly cleaned after each experiment. Cleaning the aquaria consisted of initial drainage, refilling with deionized water and then mixing the sand to resuspend any settled particles, wiping down the walls of the aquaria, draining, refilling, then repeating the above procedure and then finally refilling with 16 L of deionized water for the next experiment.

Experimental Procedure

Day 1: Acclimation

Three unionids were removed from the holding tanks and placed into a 40-L aquarium 24 hours before the first experiment. The concentration of algae to be used in the experiment the next day was added to the aquarium to acclimate the animals to the food level.

Day 2: Experiment Part I

The experimental mussels were removed from the acclimation tanks and placed into individual experimental aquaria. As each unionid was removed from the acclimation tank, it was gently agitated in the water to remove any algae, pseudofaeces and/or faeces that may have settled on the unionid or in between the zebra mussels during the acclimation period. The experimental mussels were left to acclimatize to their new environment for approximately 1.5 h or until they opened their siphons (Fig. 2).

Once the mussels were filtering, the initial or background count of the suspended particles in the water column was determined before adding any algal cells. In order to determine the particle count of aquaria water at a given time a composite sample of the water was taken with a hollow glass tube. The 8-cm long x 1-cm diam glass tube was slowly lowered into the water, sealed with pressure from the thumb and when filled to the mL marks was lifted from the aquarium. This procedure was repeated four times randomly throughout the aquarium. The total water sample of 20 mL was collected into a Coulter ‘accuvette’. This sampling procedure was repeated twice for each determination of particle concentration. Particles in a sample of water were counted using a Coulter Counter as previously described.

After determining the background particle concentration, the appropriate amount of algae was added. The algae was mixed into the aquarium by dragging the airstone back and forth across the aquarium several times for a period of approximately one minute. The air supply was left on for 2-3 minutes to allow for further mixing of the algae (initial testing proved that 2-3 minutes was long enough to mix the algae into suspension). After this time the concentration of particles in the water column was counted, as previously described to determine the number of particles (including algal cells)/mL at time equal to zero. The air supply was then turned off to prevent any faeces or pseudofaeces produced during the experiment from being resuspended and broken up. The siphoning activities of the unionids were monitored to ensure that the siphons remained open for the duration of the experiment. If a unionid closed its siphons for more than five minutes, the experiment was terminated and restarted in a clean aquarium. If the unionid's siphons remained open for 1.5 h, a final particle count was taken at this time.

During Part I of the experiment, the degree that the unionids' siphons were blocked with zebra mussels was recorded using a ranking system. Unionids received a rank of 1 when their siphons were only very slightly colonized by zebra mussels. As the amount of siphon blockage increased, the rank assigned to the unionid would increase up to a rank of 4, which was assigned to unionids whose siphons were severely obstructed by the colonization of zebra mussels. (see Appendix VII for criteria of ranking).

As well as the three experimental tanks containing infested unionids, a control experiment was conducted simultaneously. The procedure followed for the control was the same as described for the experimental aquaria, except that the control aquarium contained no experimental animals. The purpose of the control was to eliminate any errors caused by the reproduction, death or settling of suspended algae during an experiment.

Day 3: Experiment Part II

After the final particle reading in Part I, the zebra mussels attached to each unionid were carefully removed using a scalpel. The byssal threads of the zebra mussels directly attached to the unionid's shell were severed. If possible the zebra mussel colony was removed intact or in as few pieces as possible in order to minimize disturbance of the zebra mussels. The mussels from each unionid were placed into a large plastic petri dish that had been perforated in order to allow for water drainage. A unionid and its respective dish of zebra mussels were placed into a holding aquarium. The experimental concentration of algae used in Part I of the experiment was then added to this aquarium. The unionid and its separated zebra mussels were kept in this aquarium for 24 hours to allow the altered animals to adjust to their new situation. Reeders (1989) showed an increase in the filtration rate for up to 24 hours indicating progressive relaxation of the mussels in artificial circumstances.

After 24 h, the unionid and its zebra mussels were placed in an experimental aquarium. As in Part I, the animals were allowed to acclimate for approximately 1.5 h or until they resumed filtering. The experimental procedure as described for Part I was repeated for Part II using the same concentration of algae. As in Part I, a control experiment was also conducted simultaneously.

Post-Experimental Procedures

After recording the final data for Part II, the soft tissue of each unionid was dissected out and dried to a constant weight at 55°C. The zebra mussels removed from the unionids were also dried to a constant weight. The shell lengths of the individual zebra mussels were measured to the nearest micrometre with the aid of a digitizing tablet.

Calculations

In order to determine the amount of algae filtered from the water column by the unionids and the zebra mussels, the following equations were used:

Definitions of Parameters Used in Equations

B_0 ≡ Initial background particles in the aquaria water. (measured parameter)

P_0 ≡ Concentration of particles in the aquarium at time=0 immediately after the addition of the desired amount of algae. Note that this count also includes the initial dirt or background particles. (measured parameter)

P_t ≡ Final concentration of particles. This count includes both algae and background particles at time = 1.5 hours. (measured parameter)

B_t ≡ Estimated final concentration of background particles in the aquaria at the end of the experiment. (calculated parameter)

A_r ≡ Concentration of algae that was removed from the water column during the experiment. (calculated parameter)

A_i ≡ Concentration of algae that was ingested by the experimental animals. (calculated parameter)

* All measured values are the result of averaging the counts of two accuvettes (See above 'Particle Counts' for details).

Step 1. Estimate the proportion of the background particles that were not removed from suspension, assuming that background particles and algae were removed from suspension at similar rates.

$$B_t = B_0/P_0 * P_t$$

Step 2. Calculate the concentration of algae that was removed from suspension in the experimental aquaria and the control aquaria (Modified from Coughlan, 1969 See Appendix VIII).

$$A_r = \ln(P_0\text{-}B_0l)\ \text{-}\ln(P_t\text{-}B_t)$$

Step 3. The amount of algae that 'was removed' in the control aquaria was assumed to have settled during the experiment. Therefore, the amount 'removed' in the experimental aquaria minus the amount settled in the control aquaria is the amount of algae that was ingested by the experimental animals (A_i).

$$A_i = A_r \text{ experimental - } A_r \text{ control}$$

These equations were used to determine the concentration of algae ingested by the experimental animals in both Part I and Part II of the experiment.

The data generated from the above formulae were then analyzed by multiple linear regression using SAS (SAS, 1987). An orthogonal contrast was developed to estimate the amount of physical interference caused by zebra mussels. The orthogonal contrast developed to estimate physical interference was

$$Y_1 = \text{Attached - Detached}$$

Where Y_1 is the estimator of Physical interference = (The filtration rate of a unionid with zebra mussels removed from its valves but in the same aquarium) - (The filtration rate of a unionid with the zebra mussels still attached).

This contrast was used to develop a model to predict the effect of attached zebra mussels on the filtration rate of unionids for ranges of algal concentrations, degrees of siphon blockage and shell lengths of unionids.

RESULTS

A model to predict the effect of zebra mussels on the filtration rate of unionids was developed using SAS (SAS, 1987). All experimental variables including all possible interactions between the variables were initially included in the model. If a variable was not significant at the 0.05 level it was removed from the model. The least significant variable (i.e $p \geq 0.05$) was removed first, followed by the next least significant variable. Main effect variables could not be removed if they were involved in any significant interactions with the other variables. The shell length of the unionid was completely removed from the model since it was neither significant as a main effect nor was it involved in any significant interactions with the other variables. The r^2 of the model was 0.41. Concentration of experimental algae (CONC), Total length of attached zebra mussels (TLENZM), and degree of unionid siphon blockage (RANK) were all significant as main effects ($p \leq 0.01$) and all were involved in significant interactions with other parameters (See Appendix VIII for details).

The model derived for the orthogonal contrast Y_1 = Detached - Attached is:

$$Y_1 = -3.874677023 - 0.040201065*\mathrm{CONC} - 0.002200175*\mathrm{TLENZM} + 3.490790099*\mathrm{RANK} - 0.124383631*\mathrm{CONC}*\mathrm{RANK} - 0.001627365*\mathrm{TLENZM}*\mathrm{RANK} + 0.000001779*\mathrm{TLENZM}*\mathrm{TLENZM} + 0.000281110*\mathrm{CONC}*\mathrm{TLENZM} - 0.000004798*\mathrm{CONC}*\mathrm{CONC}*\mathrm{TLENZM} + 0.002323150*\mathrm{CONC}*\mathrm{CONC}*\mathrm{RANK}$$

The above equation was used to predict physical interference (Y_1) over the experimental range of the variables to illustrate the predictions of the model graphically. The RANK variable was held constant at a RANK of 2. SAS was used to estimate Y_1 (physical interference) using the other two variables in the above equation. Y_1 was predicted over the range of 0-55x10^3 cells of algae/mL for the variable CONC and 0-2500 mm of zebra mussels for the variable TLENZM. The resulting values for physical interference were plotted on a three-dimensional surface using SAS Graph (Fig. 4). This procedure was repeated to prepare graphs with RANK set at 3 and then 4. Figure (4a) illustrates the predictions of the model when the siphons are only slightly blocked (RANK = 2). The majority of points on the graph are negative values for physical interference. Therefore this graph predicts that zebra mussels interfere only slightly if at all with the siphoning activities of a unionid when the RANK is 2. Fig. 4b shows the predictions of the model when the siphons of the unionid are moderately blocked (RANK = 3). Many more of the points on the graph are positive values for physical interference. Therefore, there is more physical interference when the siphons are moderately blocked. Fig. 4c shows the prediction of the model when the siphons of the unionid are severely blocked (RANK = 4). All the values of physical interference for all concentrations and throughout the range of TLENZM predict that zebra mussels will interfere with the siphoning activities of the unionid. The largest difference between the 'attached' and 'detached' filtration rates or the largest amount of physical interference occurs when the rank of siphon blockage is the highest. The amount of physical interference also changes with the concentration of algae and total length of attached zebra mussels.

DISCUSSION

The increase in physical interference with increasing numbers of attached zebra mussels and increase in siphon blockage suggested that zebra mussels have a negative effect on the filtering activities of unionids. Although only 40% of the variability in the results could be explained by the model ($r^2 = 0.4$), there are many possible explanations. Walz (1978a) and Morton (1971) found that zebra mussels do not filter 24 h a day. In this experiment, with up to 300 zebra mussels in one experimental aquarium, it was not possible to determine how many zebra mussels were filtering during a given experiment. Since the clearance rates of Part I and Part II of the experiment were directly compared (Detached - Attached) there is a possibility that different zebra mussels could be filtering in Part I than in Part II. This inconsistency could have resulted in a large amount of variability unexplainable by the model.

Another source of variability could be the production of faeces and pseudofaeces. Ten Winkle and Davis (1982) and Walz (1978a) found that zebra mussel faeces that disintegrated rapidly into the water column caused particle counts to increase. Ten Winkle and Davis (1982) stopped their experiments whenever a zebra mussel formed faeces, but the production of faeces by a large group of mussels, as in this experiment, could not be monitored. If production of faeces increased the particle count, then the resulting physical interference would be underestimated. Therefore, production of faeces by the zebra mussels could also be a source of unexplainable variation. As with faeces, the production of pseudofaeces at the concentrations of 30,000 cells/mL and 50,000 cells/mL may also contribute to the unexplained variation. Suspension feeders produce pseudofaeces when highly concentrated suspensions cause a mechanical overload of the gills and palps (Rise and Smith, 1958). Schulte (1975) found that zebra mussels produced pseudofaeces after a critical cell density was reached. Sprung and Rose (1988) found the incipient limiting concentration for zebra mussels was 16,000 cells/mL when using monocultures of *Chlamydomonas reinhardii*. In this study, pseudofaeces production was observed for both *Dreissena* and unionids at algal concentration above the zebra mussel's incipient limiting level (30,000 and 50,000 cells/mL) but not at the concentrations below this level (10 000 and 5 000 cells/mL). Davids (1964) found that the pattern of pumping becomes very irregular in the marine mussel *Mytilus edulis* when feeding in very concentrated suspensions. He suggested this may be due to the ejection of pseudofaeces. Perhaps the production of pseudofaeces is responsible for the inflection point in the graphs around the concentration of 30,000 cells/mL.

Also, some of the variability in the filtration rates may just be due to individual variation between the experimental animals. Others that have conducted filtration studies with bivalves also report a large degree of variability in their results (Jorgensen, 1960 and Schulte, 1974 with *Mytilus*; Palmer 1980 with scallops and oysters and Rice and Smith, 1958 with the hard clam *Venus mercenaria*).

Model Predictions

The graphical illustrations in Fig. 4 show the predictions of the model over the experimental range of variables. There are large changes in the amount of physical interference between the lowest reported RANK of 2 (Fig. 4a) and the highest RANK of 4 (Fig. 4c). The variable RANK was highly significant at $p = 0.009$, suggesting that siphon blockage is important in determining the degree of physical interference caused by a colony of attached zebra mussels. The position of the attached zebra mussels, rather than the number of the attached *Dreissena*, may be the largest contributing factor to physical interference. The aperture of a unionid's siphon can be obstructed by just a few zebra mussels. It would seem logical that zebra mussels that are attached more anteriorly, away from the siphons, would not have as an large interference effect on the filtration activities of the unionid as would zebra mussels attached to the siphonal region.

Interference by Alterations in Flow

The zebra mussels attached to the unionid's siphonal region most likely interfere with the feeding currents of the unionid. Okamura (1988) reported that upstream colonies of bryozoans interfere with the feeding success of zooids downstream. She found that flow is altered as it interacts with the local topography (such as the bodies of other organisms) since slow moving eddies were formed behind the upstream colony. Therefore, the water surrounding the unionid's inhalant siphon is most likely affected by the presence of the shells of hundreds of zebra mussels attached to its valves. Okamura's results of interrupted flow by surrounding organisms illustrate how zebra mussels may be interfering with the filtering activities of the unionid host. Her results could also be used to explain why the degree of siphon blockage was significant. Buss (1979) investigated the possibility of food limitation and microcurrent interference in suspension feeders. He found that the dominant characteristics of the flow regime surrounding bryozoans are produced by the feeding activities of the organisms themselves. Through photographs of microcurrents, Buss (1979) found that when two encrusting anascan bryozoans *Onchocella alula* and *Antropora tincta* were in contact with another the clearance rate of one of the bryozoans *A. tincta* was significantly reduced. Therefore, both the physical presence of the encrusting zebra mussels and the currents they create when feeding may interfere with the filtering activities of the encrusted unionid.

Interference by Competition

This study found that when zebra mussels colonize the shells of unionids, they decrease the filtration rate of the unionid by physically interfering with the host as had been previously suggested (Lewandowski, 1976; Mackie, 1991 and Hebert et. al., 1991). Many of the unionids used in these experiments had their siphons completely blocked by zebra mussels. Attached zebra mussels protruded over the edge of the opening of the siphons. Quite often the siphons of a unionid with a RANK of 4 were not visible through the colony of attached zebra mussels. Zebra mussels crowded the siphons of the unionid, resulting in a reduced lumen of the siphon. Coughlan (1969) reported that when the marginal tentacles of the inhalant siphon were slightly turned upwards or inwards, the aperture became slightly restricted and resulted in a decreased pumping rate. Jorgensen (1960) reported that when *Mytilus edulis* were one half closed they cleared one fifth of the suspension of normally open mussels. He also stated that a small reduction in the lumen of siphons reduced the water transport by 30%. *Dreissena* attached to the edges of a unionid's valves often resulted in reduced extension of tentacles and a decreased aperture opening. The colonization of a unionid's siphonal region can therefore be classified as interference competition, since the presence of zebra mussels decreases aperture opening and subsequently decreases the filtration rate of the host unionid.

Interference competition has been reported in a wide variety of other organisms including: filter feeding marine bivalves Peterson (1980) and salt marsh snails (Brown, 1982; Stiven and Kuenzler, 1979) because of high densities; larval damselfly (Anholt, 1990), spiders (Wise, 1983) and ants (Fellers, 1987) through fitness reducing behavioral interactions and aggression; and frogs (Steinwasher, 1978) through chemical interference by secretion of growth suppressing hormones. When zebra mussels colonize the valves of unionids, it is an example of overgrowth competition, which is one of the five kinds of interference competition as described by Schoener (1983). Overgrowth competition occurs when an individual or individuals grow over or upon another individual(s), thereby depriving that individual(s) of light (as in plants) or access to waterborne food (as in sessile filter feeding animals) and possibly harming that individual by some consequence of physical contact (Schoener, 1983). There are many examples of overgrowth in the marine literature including: overgrowth of colonies of bryozoans by other bryozoans (Rubin, 1985; Okamura, 1984 & 1988; Buss, 1979; Jackson, 1977); polychetes overgrowing sea anemones (Taylor and Little, 1982); colonial ascidians overgrowing octocorals (Sebens, 1982); colonial organisms overgrowing solitary organisms (Jackson, 1977); algae overgrowing barnacles (Jernakoff, 1985; Denley and Underwood, 1979); barnacles overgrowing barnacles, (Connell, 1961; Dayton and 1971) and even tunicates overgrowing sponges which overgrew bryozoans which overgrew serpulids (Kay and Keough, 1981).

The settling of *Mytilus* on the exoskeletons of barnacles appears to be similar to the overgrowth of unionids by *Dreissena*. There are many reports of *Mytilus* settling on and overgrowing barnacles (Lively and Raimondi, 1987; Denly and Underwood, 1979; Peterson, 1979; Lubachenco and Menge 1978;

Menge, 1976; Paine, 1974; Dayton, 1971) often resulting in the death of the underlying barnacles. Peterson, (1979) and Dayton, (1971) suggested that the mussels smother and eventually starve the underlying barnacles. As with the effects of zebra mussels on the filtration activities of unionids there have been suggestions, but no concrete explanation, as to the mechanism by which *Mytilus edulis* overgrows and kills the underlying barnacles.

Dayton (1971) refers to the settlement of *Mytilus* on barnacles as organisms occupying secondary space. He states that the phenomenon of secondary space is considered relatively unimportant because barnacles do not appear to be in any danger of being eliminated by *Mytilus*. In contrast the native unionids of North America do appear to be in danger of elimination due to the effects of massive infestation by zebra mussels. Schloesser and Kovalak (1991), Mastellar and Schloesser (1992), and Gillis and Mackie (1991) have all reported significant declines in the density and diversity of unionids in the Great Lakes since the introduction of the zebra mussel. There are many endangered species of Unionidae in Great Lakes watersheds (e.g. Sydenham River (Mackie and Topping, 1988). There is a strong possibility for local extinction of these species if unionid populations continue to decline in the Great Lakes due to infestation by zebra mussels. There is even a danger of more continental declines in unionid species if zebra mussels affect Unionidae in the Mississippi River system as they have in the Great Lakes. Therefore, the effects of the invading zebra mussel on the native unionids should be further investigated to determine the mechanisms by which *Dreissena polymorpha* are impacting the native Unionidae.

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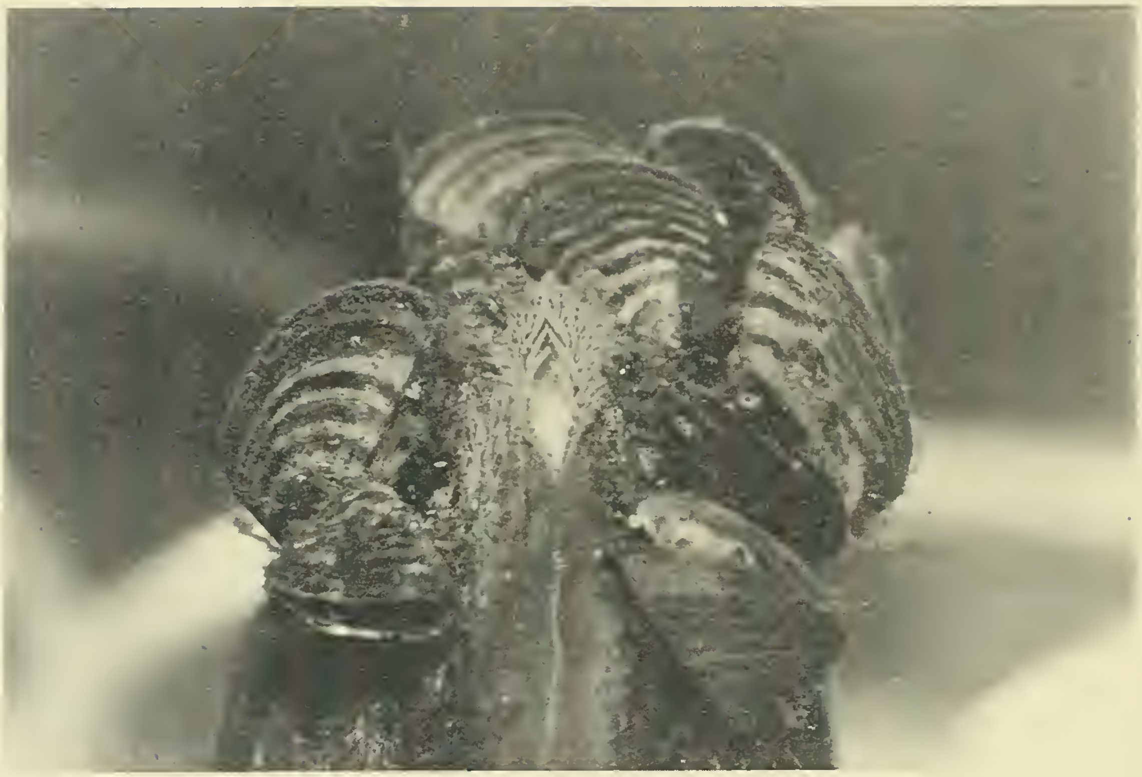


Figure 1. Proptera alata with zebra mussels attached to the siphonal region. Specimen shows an infestation of Rank 2.

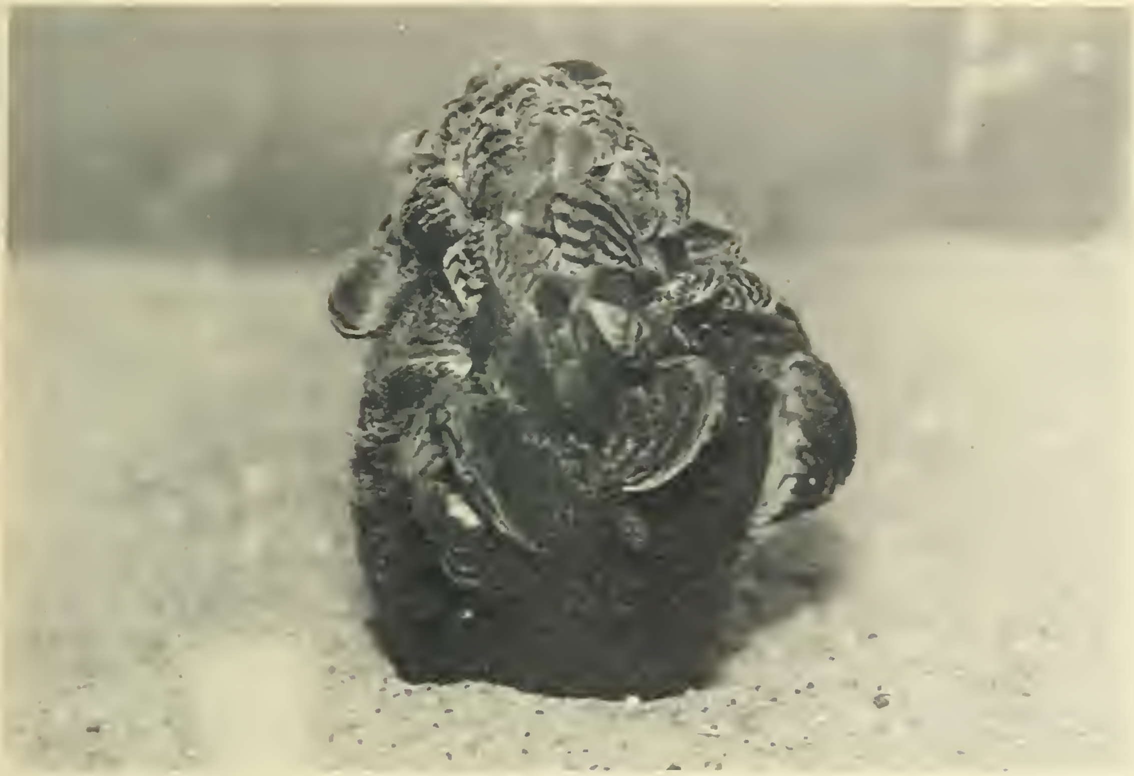


Figure 2. Proptera alata with attached zebra mussels as used in Part II of experiment. Specimen shows an infestation of Rank 3.

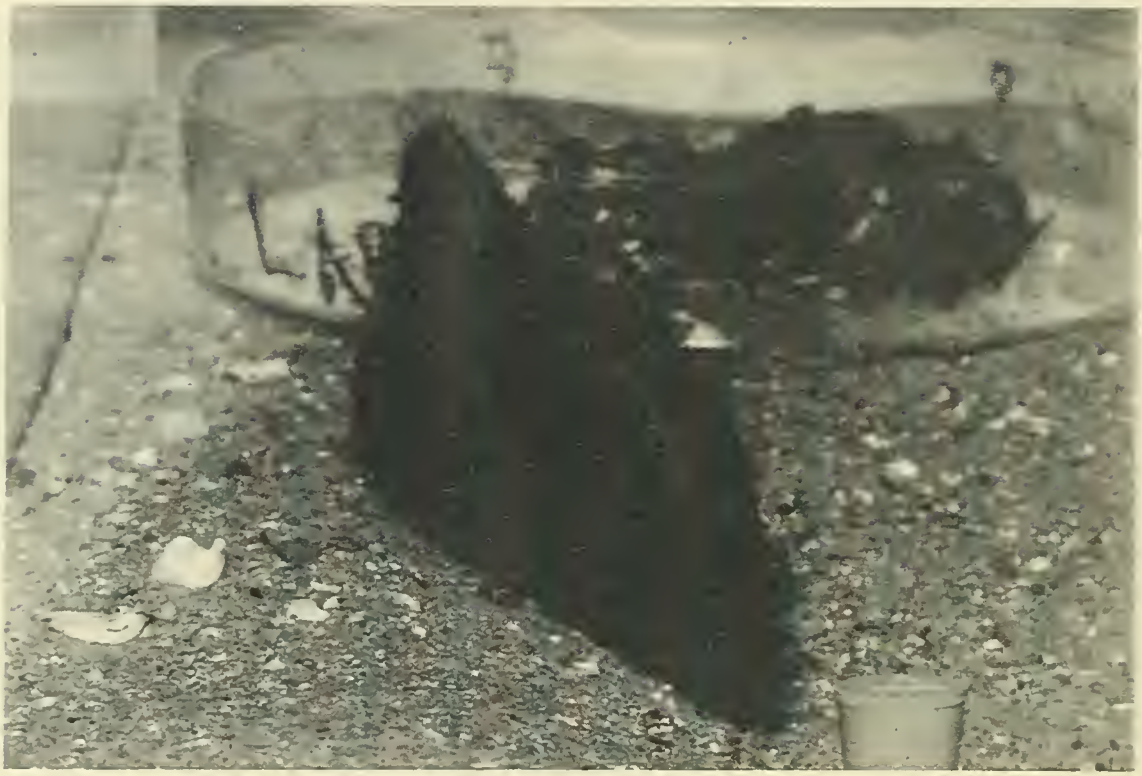
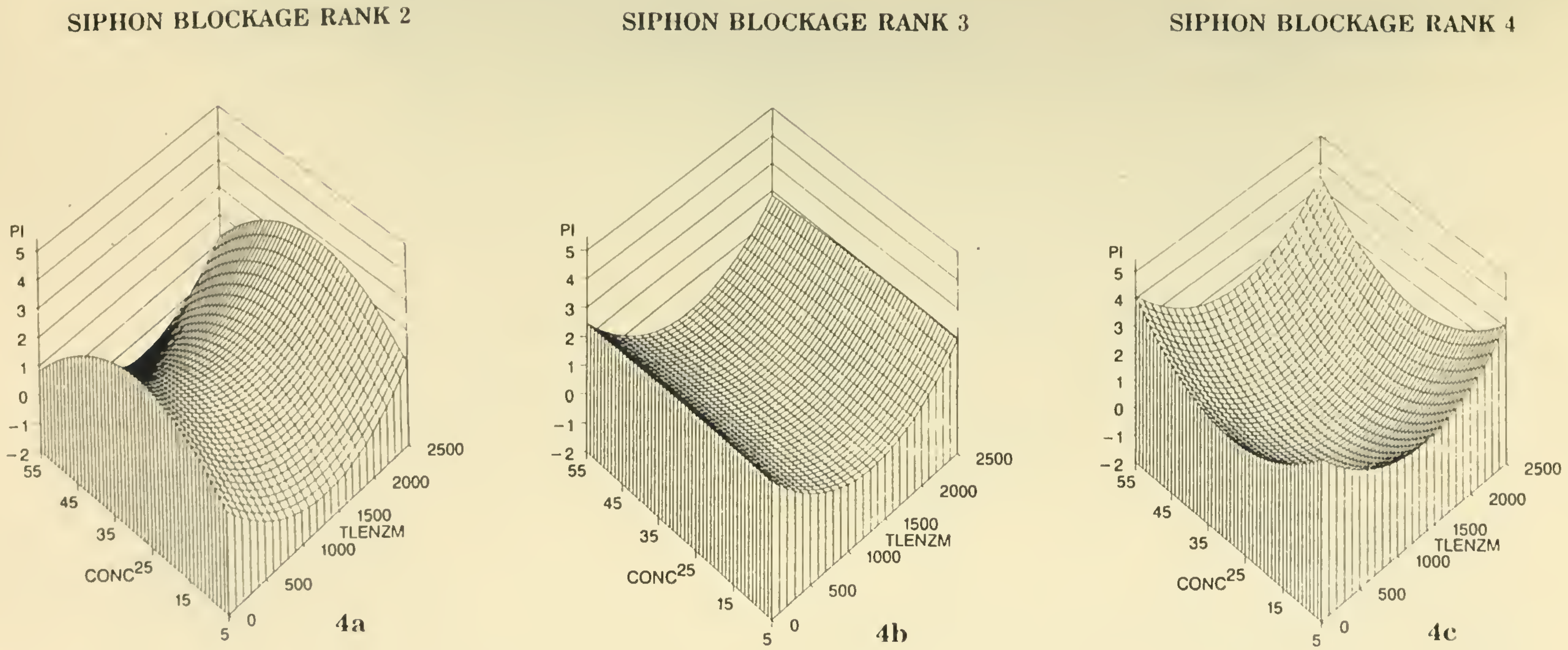


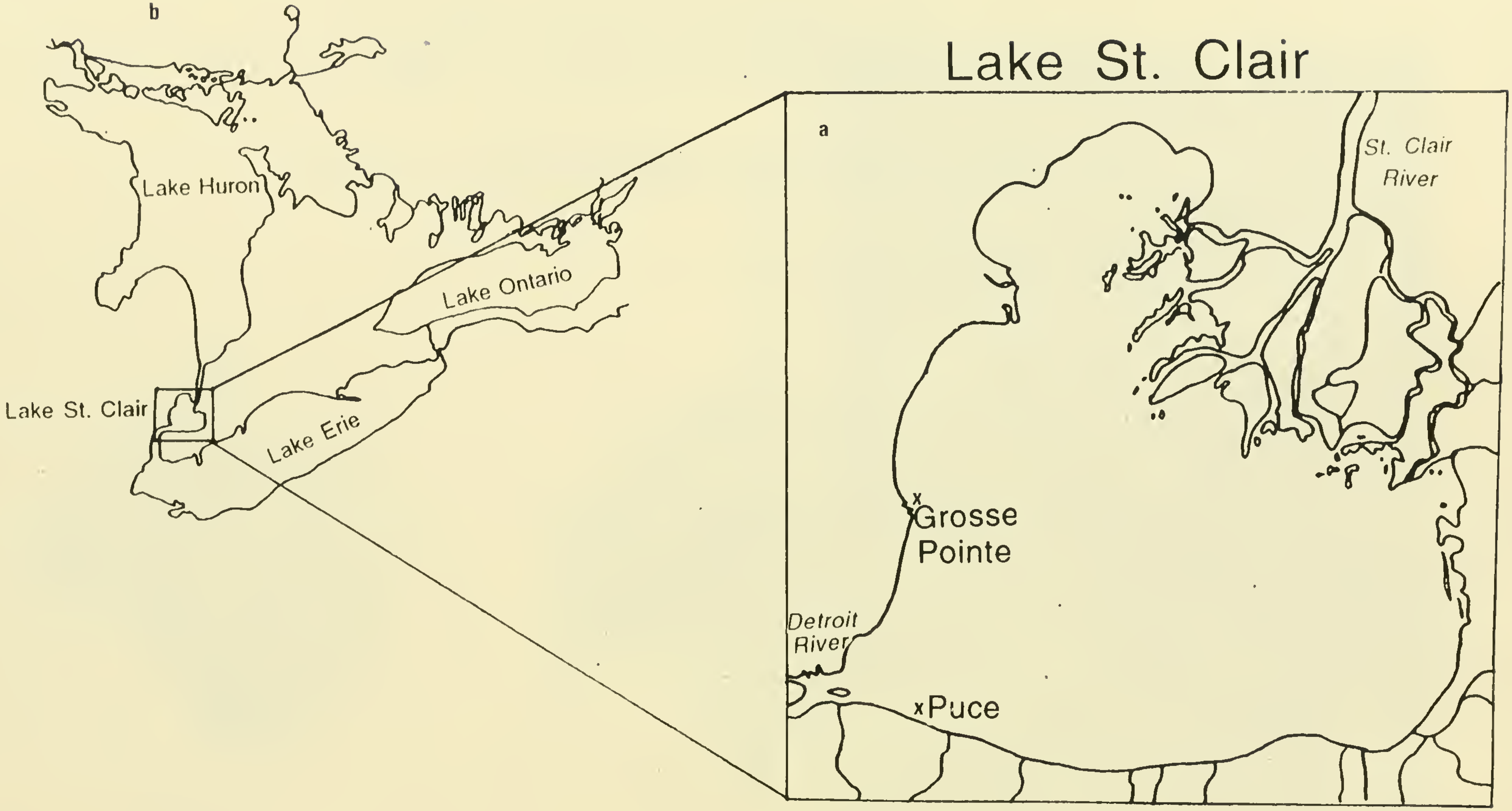
Figure 3. Proptera alata with zebra mussels removed and placed in dish in the same aquarium as used for Part III of experiment.



4a 4b 4c

Figure 4 a-c. Response of Physical Interference Model Over a Range of Siphon Blockage.

CONC = Concentration of Algae (# cells/mL x 1000) (see p. 71)
TLENZM = Total Length of Attached Zebra Mussels (mm)
PI = Physical Interference (see Appendix III)



APPENDIX I

Appendix (Ia). Map showing position of Lake St. Clair within the Great Lakes.
(Ib). Map showing the position of sampling sites within Lake St. Clair.

APPENDIX II

Appendix II . Recipe for Bolds Basal Medium (100%), the medium used to culture *Chlamydomonas reinhardii*.

STOCK	STOCK SOLUTION	ml/Litre
1. KH_2PO_4	8.75 g/500 ml	10 ml
2 $CaCl_2.2H_2O$	1.25 g/500 ml	10 ml
3. $MgSO_4.7H_2O$	3.75 g/500 ml	10 ml
4. $NaNO_3$	12.5 g/500 ml	10 ml
5. K_2HPO_4	3.75 g/500 ml	10 ml
6 NaCl	1.25 g/500 ml	10 ml
7. EDTA + KOH	10 g/L + 6.2 g/L	1 ml
8. $FeSO_4.7H_2O$ H_2SO_4	4.98 g/L + 1 ml/L	1 ml
9. BG-11 Trace Metal Mix		1 ml
10. H_3BO_3	5.75 g/500 ml	0.7 ml

* Trace Metal Mix:

Substance	g/Litre
1. H_3BO_3	2.86
2. $MnCl_2.4H_2O$	1.81
3. $ZnSO_4.7H_2O$	0.222
4. $NaMoO_4.2H_2O$	0.390
5. $CuSo_4.5H_2O$	0.079
6. $Co(NO_3)_2.6H_2O$	0.0494

-dissolve each of the above substances separately prior to adding the next on the list.

-adjust pH to approx. 7.5 (initial pH is approx. 8.5)
-when making solid media you can either add agar directly to medium or make double strength medium and double strength agar solution, then after autoclaving combine the two.
-can add 0.5 g/L hepes buffer to final medium as a buffer.

APPENDIX III

Appendix III . Summary of Unionid Sizes, Assigned Siphon Blockage Rank and Concentration of Algae Used in Each Experiment.

Unionid Identification Label	Blockage Rank	Algae Concentration	Unionid Size Class	Unionid Length (mm)	Sm	M	Lg
a	4	M	Small	92.5	1		
b	4	M	Small	89.5	1		
c	2	M	Large	133			1
e	2	L	Small	84	1		
f	2	L	Med	108.5		1	
g	2	H	Med	104		1	
h	3	H	Med	109.5		1	
k	3	H	Med	109		1	
l	4	H	Large	137			1
m	3	EL	Small	78	1		
o	3	EL	Large	124			1
p	4	M	Small	77	1		
q	3	M	Med	106.5		1	
r	2	M	Med	110		1	
s	4	L	Small	91	1		
t	2	L	Med	106		1	
u	4	L	Large	128			1
v	3	EL	Small	82	1		
w	4	EL	Small	76	1		
x	3	EL	Large	126.5			1
y	2	L	Small	76	1		
z	2	L	Med	110		1	
C	4	M	Med	105		1	
D	4	M	Large	117			1
E	4	EL	Med	97		1	
F	2	EL	Med	105.5		1	
G	2	EL	Med	106.5		1	
H	2	H	Small	92.7	1		
I	3	H	Small	75.8	1		
J	2	H	Small	92.7	1		
K	2	L	Large	120.6			1
L	2	H	Large	113.4			1
				Tally	12	12	8

	Algae Concentration			
	Extra Low	Low	Medium	High
Unionid Size Class				
Small	m, v, w,	e, s, y	a, b, p,	H, I, J,
Medium	E, F, G,	f, t, z	r, q, C	h, k, g
Large	o, x	u, K	c, D	l, L

Algae Concentration Key:

EL = Extra Low = 5 000 cells/mL

L = Low= 10 000 cells/mL

M = Medium = 30 000 cells/mL

H = High = 50 000 cells/mL

APPENDIX IV

Appendix IV. Experimental Timetable Demonstrating the Sequence of Filtration Experiments and Procedures.

Time	Day 1 (Mon)	Day 2 (Tues)	Day 3 (Wed)	Day 4 (Thurs)	Day 5 (Fri)	Day 6 (Sat)	Day 7 (Sun)
9:00	Set abc -[M]	Set del -[L]	Set ghi -[H]	Set jkl -[H]			Set mo-[EL]
9:00-1:00		Exp #1 abc-[M]	Exp #1 ef-[L]	Exp #1 gh-[H]	Exp #1 kl-[H]		Catch up
1:00		Scrape abc	Scrape ef	Scrape gh	Scrape kl		
1:00-5:00			Exp #2 abc-[M]	Exp #2 ef-L	Exp #2 gh-[H]	Exp #2 kl-[H]	
5:00-9:00			Exp # 3-M	Exp # 3-L	Exp # 3	Exp # 3	
10:00			Dry Wt. abc	Dry Wt. ef	Dry Wt. gh	Dry Wt. kl	

Time	Day 8 (Mon)	Day 9 (Tues)	Day 10 (Wed)	Day 11(Thurs)	Day 12 (Fri)	Day 13 (Sat)	Day 14 (Sun)
9:00	Set pqr -[M]	Set stu-[L]		Set vwx-[M]	Set yzA-[L]		Set CD-[M]
9:00-1:00	Exp #1 mo-[L]	Exp #1 pqr-[H]	Exp #1 stu-[L]	Catch up	Exp #1 vwx-[M]	Exp #1 yz-[L]	Catch up
1:00	Scrape mno	Scrape pqr	Scrape stu		Scrape vwx	Scrape yz	
1:00-5:00		Exp #2 mo -[L]	Exp #2 pqr-[H]	Exp #2 stu-[L]	Catch up	Exp #2 vwx-[M]	Exp #2 yz-[L]
5:00-9:00		Exp # 3	Exp # 3	Exp # 3		Exp # 3	Exp # 3L
10:00		Dry Wt. mo	Dry Wt. pqr	Dry Wt. stu		Dry Wt. vwx	Dry Wt. yz

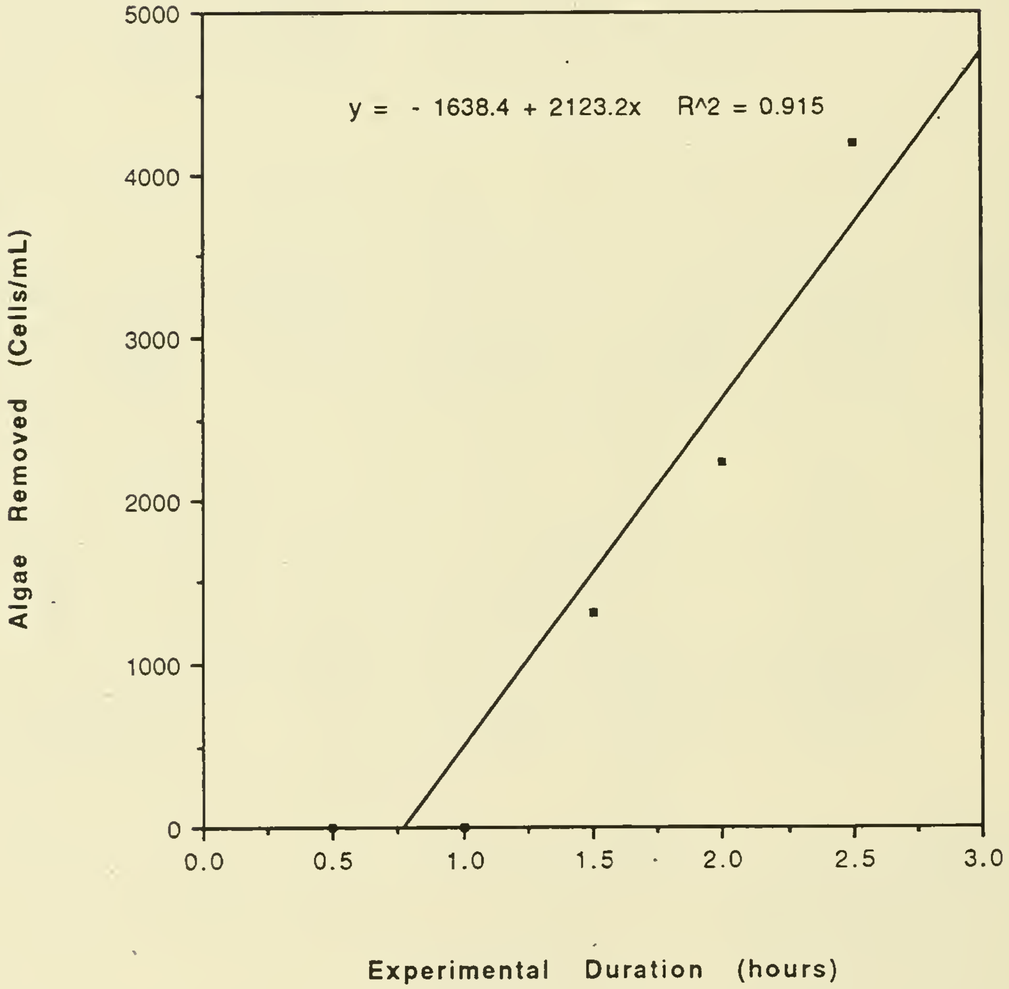
Time	Day 15 (Mon)	Day 16 (Tues)	Day 17 (Wed)	Day 18 (Thu)	Day 19 (Fri)	Day 20 (Sat)	
9:00	Set EFG-[M]	Set HIJ -[H]		Set K-[L], L-[H]			
9:00-1:00	Exp #1 CD-[M]	Exp#1 EFG-[M]	Exp #1 HIJ-[H]	Catch up	Exp#1 K-[L],L-[H]		
1:00	Scrape CD	Scrape EFG	Scrape HIJ		Scrape KL		
1:00-5:00		Exp#2 BCD-[M]	Exp #2 EFG-[M]	Exp #2 HIJ-[H]		Exp #2 KL	
5:00-9:00		Exp # 3	Exp # 3	Exp # 3		Exp #3 KL	
10:00		Dry Wt. CD	Dry Wt. EFG	Dry Wt. HIJ		Dry Wt. KL	

Key of Abbreviations:

Set = Acclimitize Unionids to Algal Concentration 24 hrs Before First Experiment
a-z and A-L = Clam Identification Label
Scrape = Remove All the Attached Zebra Mussels
Dry Wt. = Remove Soft Tissue From the Unionid to Determine the Dry Weight
Catch Up = Repeat Necessary Experiments
[EL] = Extra Low Algal Concentration = 5 000 cells/mL
[L] = Low Algal Concentration = 10 000 cells/mL
[M] = Medium Algal Concentration = 50 000 cells/mL
[H] = High Algal Concentration = 50 000 cells/mL
Exp #1 = Experiment #1
Exp #2 = Experiment #2
Exp #3 = Experiment #3

APPENDIX V

Appendix. V. Preliminary Test to Determine Experimental Duration.



APPENDIX VI

Appendix VI. Determination of Filtration Rate of a Population of Zebra Mussels Attached to a Single Unionid According to Kryger and Riisgard (1988).

Shell Length of ZM (mm)	Dry Wt of 1 ZM of this size	Filtration of 1 ZM of this size	Total number of ZM of this size	Filtration of all ZM of this size
	DW=1.54E10-5 SL^2.42	FR=aDWexp b		TL FR=#ZMxFR
1	0.0000154	0.000397011	4	0.001588044
2	8.24163E-05	0.001737305	10	0.017373051
3	0.000219864	0.004119837	32	0.131834784
4	0.000441068	0.00760238	51	0.387721374
5	0.000756882	0.01222726	76	0.929271724
6	0.001176649	0.018028248	86	1.550429286
7	0.001708669	0.025033605	73	1.827453164
8	0.00236047	0.033267721	56	1.862992353
9	0.003138973	0.0427521	34	1.453571416
10	0.004050613	0.053506015	10	0.535060152
11	0.005101419	0.065546947	15	0.983204212
12	0.006297082	0.078890915	3	0.236672744
13	0.007642996	0.09355271	5	0.46776355
14	0.009144302	0.109546088	0	0
15	0.010805916	0.12688391	5	0.634419552
16	0.012632552	0.14557826	1	0.14557826
17	0.014628746	0.165640538	1	0.165640538
18	0.016798874	0.18708154	2	0.37416308
19	0.019147163	0.209911522	1	0.209911522
20	0.021677705	0.234140255	1	0.234140255
21	0.024394472	0.259777073	2	0.519554146
22	0.027301318	0.28683091		0
23	0.030401995	0.315310337	Total # of ZM	0
24	0.033700157	0.345223594	= 468	0
25	0.037199366	0.376578613		0
26	0.0409031	0.409383044		0
27	0.044814755	0.443644275		0
28	0.048937655	0.479369449		0
29	0.053275051	0.516565486		0
30	0.057830128	0.555239089		0
		Filration rate of all zebra mussels summed		
Key:		12.6683432 litres/hour		

ZM = zebra mussels
DW = dry weight
SL = shell length
FR = filtratrion rate
a = 6.82 From Kyger & Reiisgard 1988
b = 0.88 From Kyger & Reiisgard 1988
TL FR = total filtration rate

Dry weight (column 2) is in grams. Filtration Rate (column 3) of a single mussel of size in column 1 is litres/h, and Filtration rate of all mussels (column 5) is litres/h.

APPENDIX VII

APPENDIX VII . The Criteria for Ranking the Degree of Unionid Siphon Blockage

Rank	Description
1. Clear of Zebra Mussels	- can extend tentacles fully (100%)* - siphon is able to open fully, not blocked (0%) - No ZM attached to edge of siphons
2. Slightly Blocked	- mostly (75-100%) able to extend tentacles - siphonal opening is slightly (0-25%) blocked - 1 or 2 ZM attached to siphon area
3. Moderately Blocked	- moderately (50-75%) able to extend tentacles - siphonal opening is moderately (25-50%) restriction - 3 or 4 ZM attached to siphon area
4. Extremely Blocked	- only slightly (0-50%) able extend tentacles - siphonal opening is very (50-100%) restricted - 5+ ZM attached to siphon area

* Percentages are amounts (eg. of amount of tenticle extension) relative to an uninfested unionid.

1----------4

Clear--------Blocked

APPENDIX VIII

Appendix VIII . Adaptations to Coughlan's (1969) Filtration rate equation.

Equation for calculating Filtratraion rate from clearance rate Coughlan (1969)

$$m = M/n\ [(\log_e C_0 - \log_e C_t)/t - a] \qquad (1)$$

Where

m ≡ Filtration rate

M ≡ Volume of suspension

n ≡ Number of animals

C ≡ Concentration of suspension

a ≡ Rate at which particles settle out of suspension

$$a = (\log_e C_0' - \log_e C_t')/t \qquad (2)$$

Where

C_0' ≡ Concentration of suspension at time=0 for the control aquarium.

C_t' ≡ Concentration of suspension at time=t for the control aquarium.

Therfore incorporating (2) into equation (1) becomes:

$$m = M/n[(\log_e C_0 - \log_e C_t)/t - (\log_e C_0' - \log_e C_t')/t] \qquad (3)$$

The equation used in this study:

$$FR = A_t\text{experimenatal} - A_t'\text{control} \qquad (4)$$

Which in full is:

$$FR = [(\ln P_0 - B_0) - (\ln P_t - B_t)] - [(\ln P_0' - B_0') - (\ln P_t' - B_t')] \qquad (5)$$

Where

B_0 ≡ Initial dirt or background particles in the aquaria water. (measured)

P_0 ≡ Concentration of particles in the aquarium at time = 0 immediately after the addition of the desired amount of algae. Note this count also includes the initial dirt or background particles. (measured)

P_t ≡ Final Concentration of particles. This count includes both algae and dirt particles at time = 1.5 hours. (measured)

B_t ≡ Final concentration of dirt or background particles in the aquaria at the end of the experiment. (calculated)

A_r ≡ Concentration of algae that was removed from the water column during the experiment. (calculated)

A_i ≡ Concentration of algae that was ingested by the experimental animals. (calculated)

Adaptations from Coughlan (1969)

1. All expeiments in the study were conducted in 16 L of water, therefore M = 16 L. The duration of all experiments in this study was 1.5 hours, therefore t = 1.5. Therefore, the data were normalized for 16/1.5.**

2. The number of zebra mussels (n from equation 1) was not used in this experiment because the zebra mussels attached to one unionid were considered a as a group not as individuals. The total length of all attached *Dreissena* was used as a variable in the physical interference model.

3. Since sand was used as a substrate in this experiment the aquaria water was not filtered to remove

background particles before use because particles would just become resuspended when the experimental water was poured into the aquarium. Therefore, the equations were modified to account for any background particles. Equation 4 contaains variables B_0 and B_t that were not used by Coughlan (1969) equation 1. Thus, the measurement of B_0 (the background particles at $t=0$ and the calculation of B_t (the estimated background particles at $t = 1.5$ hours.

APPENDIX IX

Appendix IX. Details of experimental model developed using sas SAS to determine if zebra mussels are physically interfering with the siphoning activities of unionids.

```
Dependent Variable: Y1
                               Sum of                 Mean
Source               DF        Squares                Square       F Value     Pr > F
Model                7         16.39248767            2.34178395   2.37        .05389
Error                24        23.66786233            0.98616093
Corrected Total      31        40.06035000

          R-Square C.V.                   Root MSE          Y1 Mean
          0.409195 453.9686               0.993056          0.21875000


Source               DF      Type III SS          Mean Square         F Value       Pr > F
CONC                 1       8.30348987          8.30348987            8.42        .00783
TLENZM               1       7.85225508          7.85225508            7.96        .00944
RANK                 1       7.93287462          7.93287462            8.04        .00913
CONC*RANK            1       9.84895606          9.84895606            9.99        .00423
CONC*CONC*RANK       1      11.07636386         11.07636386           11.23        .00266
TLENZM*TLENZM        1       6.47336398          6.47336398           6.56         .01711
CONC*CONC            1       9.56741889          9.56741889            9.70        .00472


Dependent Variable: Y1

                                               T for H0:          Pr > |T|     Std Error of
Parameter                          Estimate                 Parameter=0        Estimate

INTERCEPT                      -3.581921088       -1.77       .08931        2.02286846
CONC                            0.604962172        2.90       .00783        0.20848355
TLENZM                         -0.003670838       -2.82       .00944        0.00130089
RANK                            2.042300584        2.84       .00913        0.72007588
CONC*RANK                      -0.200331321       -3.16       .00423        0.06339101
CONC*CONC*RANK                  0.003503674        3.35       .00266        0.00104544
TLENZM*TLENZM                   0.000001369        2.56       .01711        0.00000053
CONC*CONC                      -0.010616278       -3.11       .00472        0.00340839
```

Key: CONC = concentration of algae used in the experiment
TLENZM = Total length of zebra mussels attached to the experimental unionid
RANK = Degree of siphon blockage

DISTRIBUTION, GROWTH AND CONTAMINANT UPTAKE BY ZEBRA MUSSELS (DREISSENA POLYMORPHA) IN ONTARIO SURFACE WATERS

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SUMMARY

This study assessed the dispersal rates, growth rates (both vertically in a water column and horizontally from one lake to the next), and population sizes of zebra mussels (Dreissena polymorpha). Five sites along the Rideau Waterway, three sites on the northeastern shores of Lake Ontario, and nine sites along Georgian Bay and the North Channel of Lake Huron were monitored throughout the summer of 1991 for veligers and adults to determine dispersal rates and selected population dynamics. Neither adults, larvae, or settled juveniles were found at any of the sites examined on the Rideau Waterway, nor on Georgian Bay or the North Channel. Small numbers of veligers were seen in some samples from the Lake Ontario sites. Ropes strung off coast guard navigation buoys in Lake Ontario, Lake Erie, and Lake St. Clair yielded very few newly settled juveniles, and many ropes were lost due to wave action. Zebra mussels attached to anchors, chains and buoys of coast guard navigation buoys were examined to determine (1) the variations in sizes of mussels with depth and (2) the vertical distribution of contaminants in the Great Lakes. During the decommissioning of 3 buoys from the western basin of Lake Erie in November, 1991, mussel samples were removed from various depths along the chains, and were analyzed for growth parameters, and for various organic (total chlorobenzenes (tCB), polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs)) and inorganic contaminants (cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), and lead (Pb)). Length-frequency data suggest growth decreases with increasing depth (maximum 9 m), possibly due to lower dissolved oxygen levels recorded at these deeper depths. One to three cohorts appear to occur at several depths but the deepest depths (about 9 m) appear to have only a single cohort of small individuals. PCB, Cd, Ni, and Cu concentrations in mussels appear to vary with depth, but the lack of replicate samples from some depths did not allow for statistical comparisons. However, the data suggest that contaminant distribution, possibly of biologically available forms, appears to vary significantly with depth, even within the epilimnion (i.e. down to the 9 m depth). PAH- and Zn concentrations appear to show no distinct

trends with depth. Chlorobenzenes and lead (with the exception of one sample) were not detected in zebra mussels.

INTRODUCTION

The recent introduction of the zebra mussel (Dreissena polymorpha) into the Great Lakes is proving to have major ecological and economic impacts, particularly in Lake Erie and Lake St. Clair where the mussels were originally introduced. Information on the dispersal rates of veligers and adults into other Great Lakes not yet invaded by the zebra mussel, as well as into major waterways off the Great Lakes, and the growth rate of the new populations would help to model predictions of rate, extent and size of ecological and economic impacts in the newly-invaded bodies of water. This information would be particularly useful to industries which are interested in predicting the time of invasion of zebra mussels into their location, and to the potential impact they might have on operations when the mussels arrive.

Several studies have outlined the vertical distribution of contaminants in the water column (Baker and Eisenreich 1985; Baker et al. 1991), but none have examined the vertical distribution of contaminants using benthic organisms, mainly because few, if any, native species of benthos have the ability to grow naturally in a vertical plane within the water column. Zebra mussels growing naturally along the entire length of coast guard navigational buoys and chains offer a unique opportunity to examine the vertical distribution of contaminants in the Great Lakes. The zebra mussel may be a potentially useful biomonitor for the monitoring the vertical distribution of contaminants.

The objectives of this study are: (1) to determine the dispersal rate of zebra mussels into previously non-invaded areas of the Great Lakes; (2) to determine the rates of growth and sizes of populations of adults and larvae in the new habitats; (3) to determine the effects of depth on abundance, biomass, and growth rates of zebra mussels; (4) to estimate the biomass of zebra mussels on navigational buoys and their chains in different areas of the Great Lakes; (5) to assess the potential of zebra mussels for monitoring the vertical distribution of contaminants within the Great Lakes.

MATERIALS AND METHODS

During the summer and fall of 1991, bimonthly samples were taken to determine zebra mussel presence at various locations along the North Channel, Georgian Bay, Lake Ontario, and the Rideau Waterway from Lake Ontario to Ottawa (Fig. 1). The bimonthly collection for each site consisted of 6

Schindler-Patalas trap (12-L) samples for veligers, and 1 settlement rack sample containing 5 coupons (plexiglas plates approximately 13 cm x 5.5 cm each) for detecting settlement stages of postveligers and drifting juveniles or translocating adults. Veliger samples were preserved in 70% ethanol, while the settlement coupons were allowed to air dry. Veligers and adults for each sample date and location were counted in order to determine dispersal rates between sites, as well as growth rates and abundance of juveniles and adults.

To determine the effects of depth on the growth rate and abundance of zebra mussels, 1.58-cm (0.625-in) diameter polypropylene ropes were strung off coast guard buoys (2 per buoy) in the following locations: 5 buoys in the western basin of Lake Erie, 3 buoys in Toronto Harbour, and 2 buoys near Amherst Island in eastern Lake Ontario (see asterisks, Fig. 1). Deployment, in mid-June, 1991, was such that the bottom of each rope was approximately 1 m off the bottom, and kept taut by a small brick tied to the end. One rope in Lake St. Clair was also separately deployed in June using a buoy and anchor, and was retrieved in October, 1991. The coast guard buoy ropes were also intended to be retrieved at this time, but this was not possible, as explained in the results section. The intention of this procedure was to collect mussels that settled along the length of the rope during the summer of 1991 (i.e. short-term trends) and compare the growth and abundance of mussels with those on chains of adjacent navigation buoys that had mussel accumulations of one to several years (i.e., long-term trends). The chains examined were those that the Canadian Coast Guard leaves in place every year and replaces only the buoy with a winter spar.

For studies of the effect of depth on growth rate of mussels and contaminant levels in zebra mussels, random samples of zebra mussels were taken from three navigation buoys and their chains and anchors that were decommissioned in November, 1991 for the winter. Two of the buoys (with chains and anchors) were from the western basin of Lake Erie in Pelee Passage (buoy X and P) and one was from Toledo Harbour, Ohio (buoy 10) (Fig. 1). These particular buoys and chains are removed every year; therefore samples were representative of only one season's settlement and growth. Sampling consisted of removing mussels at 1-m intervals along the buoy and chain, obtaining a sample for both growth and abundance analysis, as well as for contaminant analysis. In addition, during August, 1991, water temperature and dissolved oxygen readings were taken throughout the water column for locations adjacent to buoys X and P. No such samples were taken for the Toledo Harbour buoy.

Sampling of the buoys and chains after winter decommissioning posed some problems. Uneven mussel distribution along the length of the chains prevented the sampling of all depth increments for each buoy. The patchy distribution of mussels at some depths only provided sufficient numbers for contaminant analyses. On the other hand, other depths had large numbers of mussels over greater lengths of the chain and there were sufficient numbers to allow for studies of both contaminant levels and size distribution (Fig. 2). In addition, the anchors and chains of the two Pelee Passage buoys were separated from the buoys themselves. However, during the decommissioning process, the coast guard personnel did not record which anchor belonged to which buoy and could only guess at their identities. Hence the identity of the anchors are still in question. The results present data from anchor 1 with buoy P, and anchor 2 with buoy X, with further explanations given in the discussion.

Contaminant analysis was performed by Ortech International in Mississauga, Ont. Specific contaminants of interest were chlorobenzenes (CBs), total polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). Each sample for the contaminant analysis consisted of a random group of mussels in a size range of 10 to 15 mm; the 9-m depth sample for Pelee Passage, buoy X, fell outside this range, and were 5 to 13 mm. Shells were shucked and approximately 6 to 7 g of wet tissue per sample was used for the organic chemical analysis and approximately 2 g of wet tissue per sample was used for the metal analysis. Analytical procedures are outlined in Appendix I. All data for PCBs were well above the 5% detection but below the 5% detection limit for tCBs and PAHs; Ortech considers data above the 5% detection limit more reliable than data below this value.

RESULTS

Dispersal rate of zebra mussels

Upon analysis of the Schindler-Patalas trap and settlement rack samples for all locations and dates, no larvae or adults were found. Therefore, although Ojectives 1 and 2 were completed, the results indicate that dispersal rates north into Lake Huron, Georgian Bay and Lake Superior were either negligible or conditions were not appropriate for reproduction or settlement at magnitudes sufficient to be detected by our sampling methods (e.g. less than 1 larvae in 72 L, (i.e. 6, 12-L Schindler-Patalas trap samples). Similarly, dispersal into the Rideau River system appears to be slower than anticipated. Monitoring of some of the same sites was performed in 1991 by the Ontario Ministry of Natural Resources and no zebra mussels were found by them. However, they did find zebra mussels on the Trent Waterway (e.g. Rice Lake, Balsam Lake, Buck Lake) in 1991 (Lois Deacon, OMNR, pers. comm.). Zebra mussels were sighted in Georgian Bay at Owen Sound Harbour for the first time in 1992 (Dr. Dave Payne, OMNR, pers. comm.). It is surprizing that zebra mussels were not found at the sites on Lake Ontario (Cobourg, Bayside, Millhaven, Fig. 1) but water currents, which tend to flow along the U.S. shores, may be slowing the dispersal rate of zebra mussels along the Ontario shores of Lake Ontario. Therefore, it appears that dispersal rates northward (from Lake Erie and Lake St. Clair) and inland via the Rideau River system are indeed rather slow.

Abundance and growth rates of zebra mussels in relation to depth

The mechanical design of the ropes strung off the various buoys proved to be largely ineffective against the strain posed by the buoys, as most ropes were worn through upon checking after 2 months of deployment. For the ropes that did remain, there was little or no settlement and as a result, no reliable growth data in relation to depth were obtained using this method. The small settlement (less than $50.m^{-1}$) is not surprising because there was little settlement in Lake St. Clair in 1991 (Mackie, 1992), possibly due to higher than average summer temperatures.

It appears that zebra mussels are able to survive at all levels in the water column, at least for the depths (down to 9 m) examined in this study. The average sizes of mussels tends to decrease with increasing depth. For example, at Toledo Harbour mean shell length (of total sample) decreased from 10.5 mm at 1 m, to 8.5 mm at 4 m and 6.3 mm at 9 m (Fig. 3); at Pelee, Passage (Buoy x) from 12.5 mm at 1 m to 11.0 mm at 2 m and 3.8 mm at 9 m (Fig. 4); and at Pelee Passage, Buoy P from 12.8 mm at 1m to 10.8 mm at 4 m (Fig. 5). Dissolved oxygen measurements taken for the Pelee Passage buoys in mid-summer decreased only slightly with increasing depth, while temperature remained relatively constant throughout the water column (Table 1). The average shell length of mussels at depths of 4 m and less for the Pelee Passage buoys appears to be higher than that for the Toledo buoy, while the opposite was true at the 9 m depth (Figs. 3 - 5). A bimodal to trimodal length-frequency distribution appears to occur at some depths, in particular the 1 m, 2 m, and 4 m depths for the Pelee Passage buoys (Figs. 4 and 5) and the 1 m and 4 m depths for the Toledo Harbour buoy (Fig. 3). A unimodal length-frequency distribution appears to be present at the 9-m depth for both the Pelee Passage and Toledo harbour buoys.

Total PCB, PAH and CB contaminant levels in zebra mussels in western Lake Erie

There appears to be variations in zebra mussel concentrations of PCBs with depth (Fig. 6), while the variations in concentrations of other organic contaminants are less pronounced (Fig. 7). Additional analyses are needed to determine if the variations with depth are statistically significant. Nevertheless, it appears that PCB concentrations tend to increase at the 2 to 4 m depths for buoy P and the Toledo buoy respectively, while steadily decreasing with buoy X (Fig. 6). PAH concentrations in general appear to remain relatively constant at all depths for both sites (Fig. 8). All samples collected for PAHs were either below the detection limit, or < T. CBs in all samples were below the detection limit.

Similarly, there appear to be variations in zebra mussel tissue concentrations of some inorganic contaminants (e.g. Cu, Fig. 8; Cd, Fig. 9) in relation to depth, while with others (e.g. Zn, Fig. 11) the variations in concentrations with depth are less pronounced, mainly due to large errors around the means (note that all metal concentrations are reported on a wet weight basis). At Toledo Harbour, there appear to be increases in levels of metals, especially Cu, and Cd (Figs. 8 - 9) at the 2 m and 6 m depths; similar trends are apparent with Zn but the errors around the means are large (Fig. 11). At Pelee Passage buoy P the levels of metals appear to increase only at the 3 to 4 m depth with considerably lower levels at the surface and bottom of the lake (Figs. 8 - 11). At buoy X values show no distinct trends due to large errors around the means. With the exception of a relatively large value of 4.6 ppm at the 4 m depth on buoy P, all lead levels are below 0.5 ppm for the Pelee Passage samples, and below 1 ppm for the Toledo samples. For this reason Pb data was not included here.

In general, concentrations of most contaminants seem to be somewhat higher at the Toledo Harbour site than for the Pelee Passage sites (Figs. 6 - 11).

DISCUSSION

Sampling from buoys and chains after winter decommissioning is not an effective means for collecting zebra mussel growth data. Firstly, the handling of the heavy buoys and chains during the decommissioning dislodges several mussels, while crushing many of those mussels which remain attached. Consequently, it is difficult to obtain appropriate samples for all depths and for some (but not all) samples, there may be large standard errors for abundance, length-frequency distributions, and biomass. Secondly, there are potential problems associated with keeping components together during the decommissioning procedure; for example, on board ship, the buoy and chain/anchor are separated from one another, thereby creating problems as to which buoy belongs to which chain/anchor. Overall, a more appropriate means of sampling for depth distribution would be to remain on board the ship itself and remove samples as the buoy is decommissioned, or preferably collect samples via SCUBA while the buoy is still operational in the water.

In fact, this problem arose during the study. During the decommissioning procedure, the anchors were separated from the chains without noting which anchor belonged to which chain. It was surmised that anchor 1 belonged to buoy P because the results of the contaminant data fit the trend for the water column. Samples from buoy P (closer to the Detroit River) had higher levels of PCBs than samples from buoy X (further away from the Detroit River). Similarly, anchor 1 samples had higher PCB concentrations than anchor 2 samples.

The relationship between decreasing size (shell length) of zebra mussels and increasing depth may be a result of both chemical and physical differences with depth. Water temperature remained relatively constant over the 9 m water depth and was probably not a factor in explaining the decreasing size of mussels with increasing depth. Dissolved oxygen levels decreased slightly, and may be a factor in at least partly explaining the size distribution of zebra mussels. Chlorophyll a levels were not measured, but data from MacIsaac (pers. comm.) suggest that Chlorophyll a levels do not change significantly in the first 9 m of water. Light intensity, if a factor, would be indirect through its affect on Chlorophyll a.

The differences in sizes of mussels at the same depths for the Pelee Passage and Toledo Harbour sites may be due to differences in temperature and/or food abundance (measured as chlorophyll a content) for the various depths at these two locations. Studies by Shevtsova and Kharchenko (1982) showed a larger biomass of zebra mussels with higher levels of seston between sites in the same body of water. Future investigations should therefore include these measurements to test this hypothesis for the Great Lakes.

The bimodal to trimodal length-frequency distributions observed for some depths would indicate one to three cohorts for these locations. This suggests that postveliger settlement is more extensive in the 1 to 4 m depths than at the 9 m depth. The data further suggest that most of the settlement occurs in the first 4 m of water; perhaps only a few postveligers sink to the 9 m depth to settle. This may also explain, in part, the smaller average size with depth; it is possible that the slower-developing veligers settle out last, and deeper, and this slower settling rate and development rate may explain the smaller sizes at deeper depths. Additional analyses of the present data and additional sampling will be required to quantitatively determine this.

The value of 4.6 ppm for lead at the 4 m depth on buoy P is quite unusual, considering that all other samples contained very low concentrations of this metal. This suggests that there may have been contamination during the collection/analysis procedures.

The data suggest that there may be an influence of depth on the concentrations of some contaminants in zebra mussels, particularly for PCBs and some metals. The changes with depth will best be explained when comparisons with data for ambient concentrations of these contaminants in the suspended solids and water are performed, as well as increasing the amount of replication so that statistical comparisons can be made. This information is particularly important because differences in tissue concentrations with depth may be a function of environmental variables (e.g. temperature) affecting the uptake by the mussel, rather than a reflection of the absolute ambient concentration of the contaminant itself. For example, the unstratified waters of the western basin may well have a homogeneous distribution of contaminants in the water column, but perhaps food sources vary with depth. This may affect growth rates and possibly uptake rates of contaminants. Future investigations should therefore include these types of data.

Overall, it appears that Toledo Harbour is a more contaminated area than the Pelee Passage. This would indicate that there are point source inputs in or around the Toledo area. This may also be due to the effects of three main water flow masses that feed into the western basin from the Detroit River (Kauss and Hamdy 1985). The western shoreline flow mass from the Detroit River, which probably transports contaminants to Toledo, will have less of a dilution effect on the contaminants than that of the central, larger flow mass, which would transport contaminants to the Pelee Passage. The concentrations in Toledo may be higher because of this.

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Table 1. Temperature and Dissolved Oxygen values in relation to depth in the Pelee Passage (buoy P and X), Lake Erie, Ont.

depth (m)	Buoy P temp. (°C)	Buoy X temp. (°C)	Buoy P, D.O. (ppm)	Buoy X, D.O. (ppm)
1	22	24	8.0	7.9
2	22	24	8.1	7.8
3	22	23.5	8.0	7.8
4	22	23.5	8.0	7.8
5	22	23.5	7.8	7.8
6	22	23	7.6	7.6
7	22	23	7.6	7.7
8	22	23	7.6	7.6
9	22	23	7.6	7.6
10	22	23	7.5	7.6

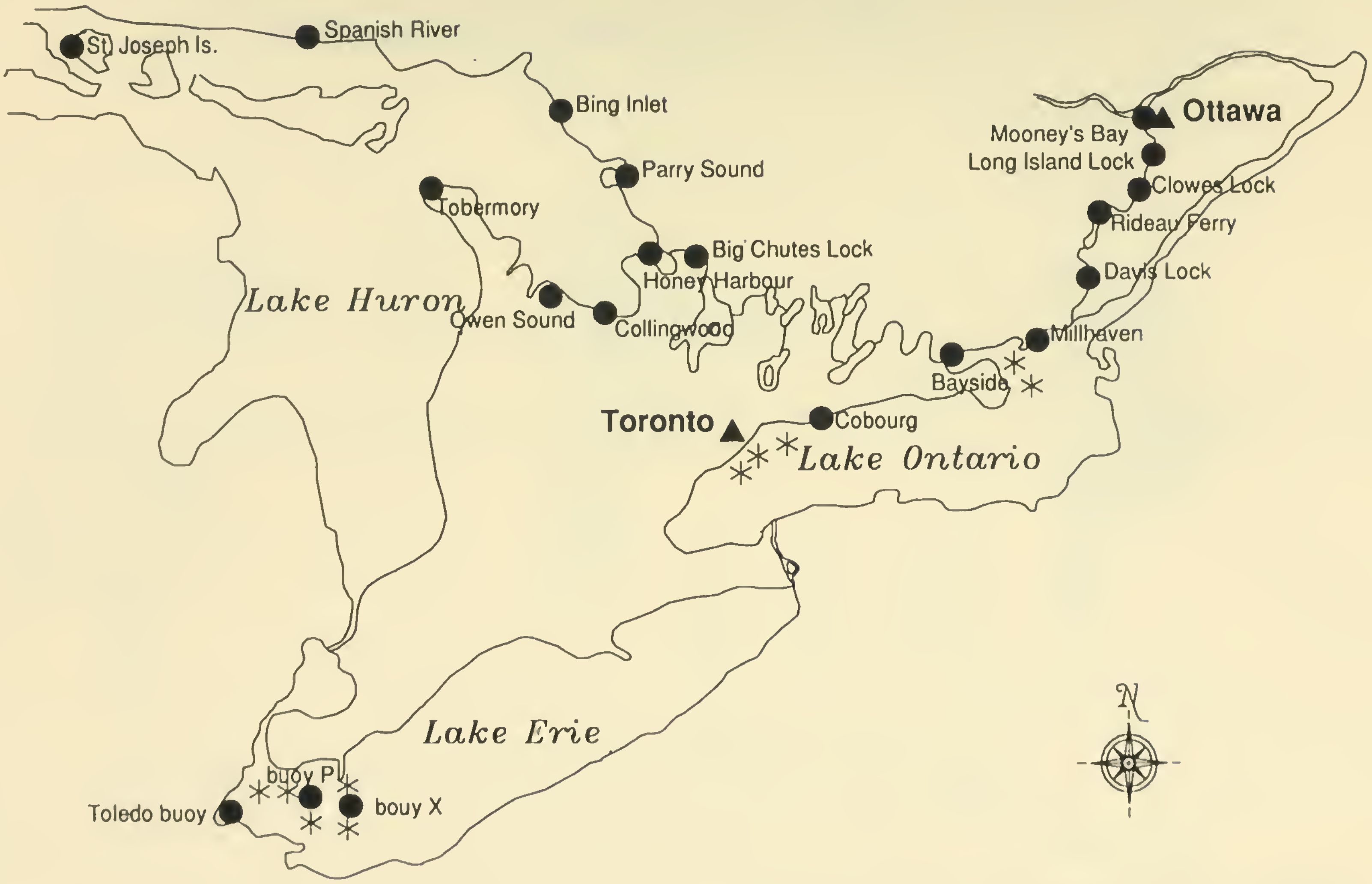


Fig. 1. Locations of sites in 1991.

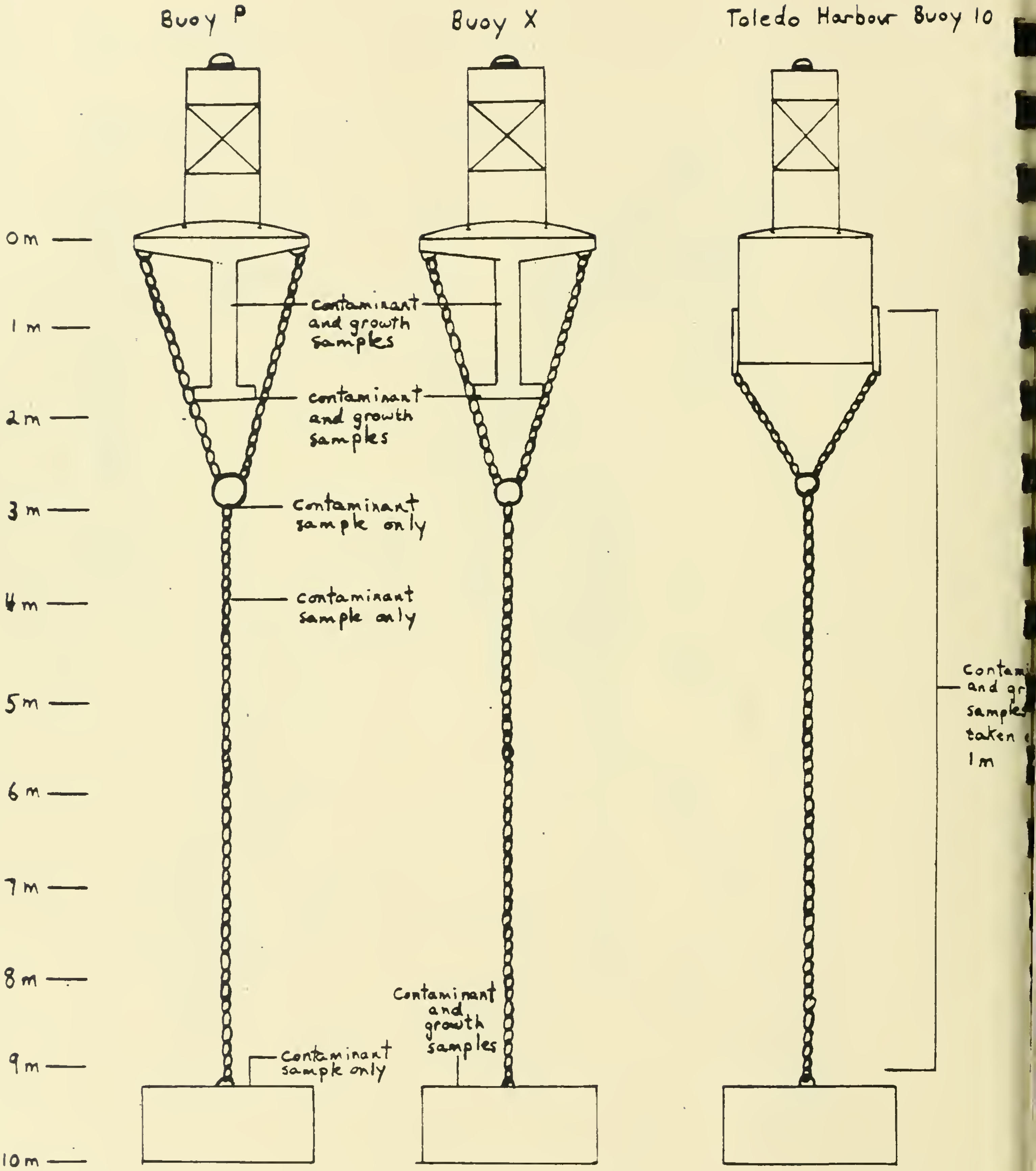
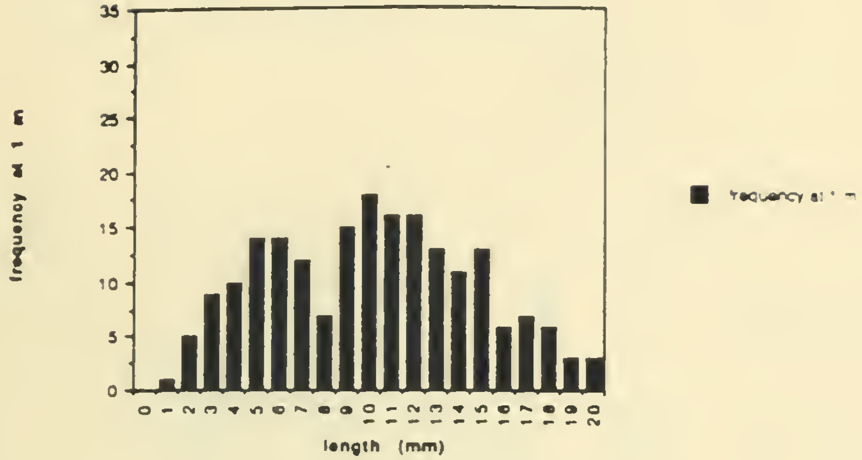


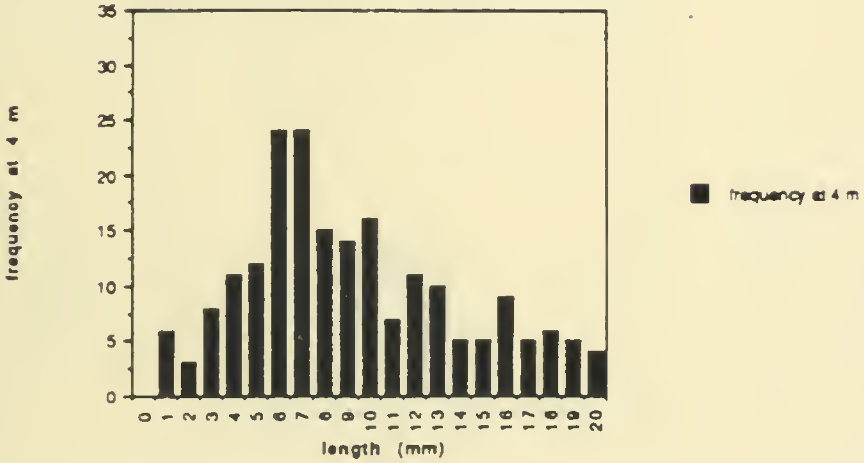
Fig. 2. Locations on buoys, anchors, and chains where growth and contaminant samples were taken (diagram not to scale).

Fig. 3.

Length/frequency distribution of zebra mussels at the 1 m depth in Toledo Harbour, Ohio.



Length/frequency distribution of zebra mussels at the 4 m depth in Toledo Harbour, Ohio.



Length/frequency distribution of zebra mussels at the bottom (9 m) in Toledo Harbour, Ohio.

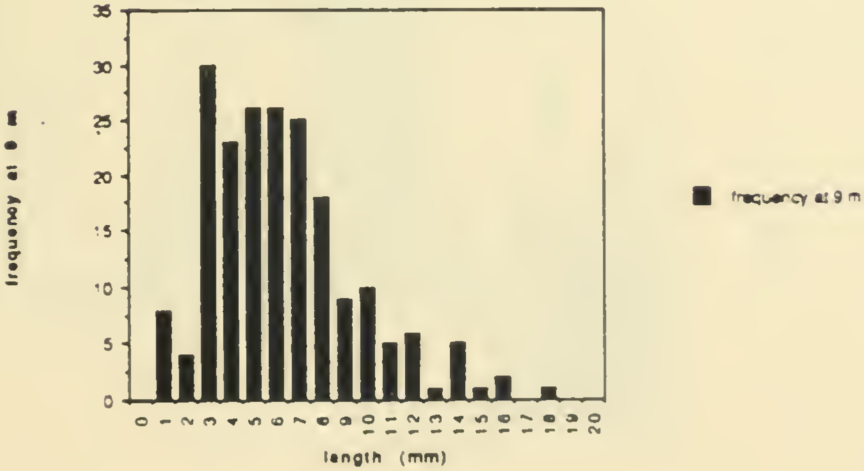
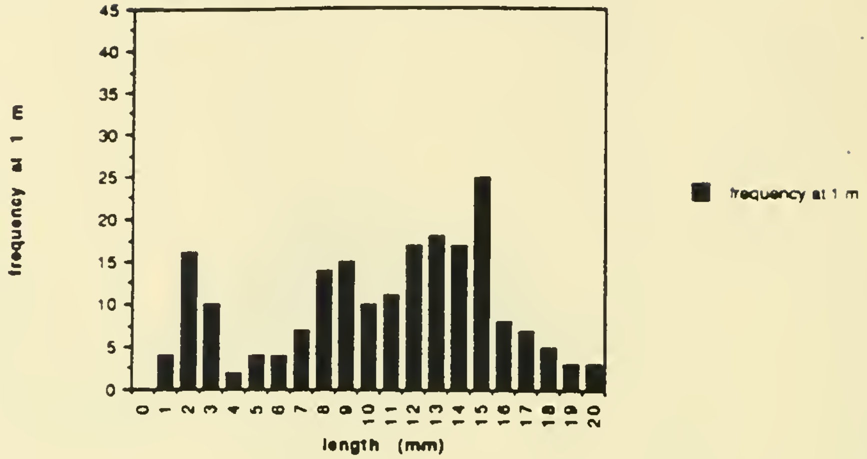
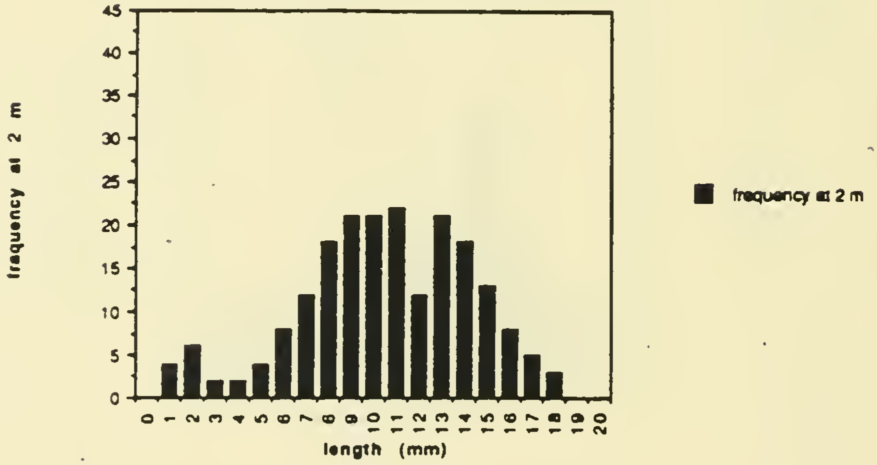


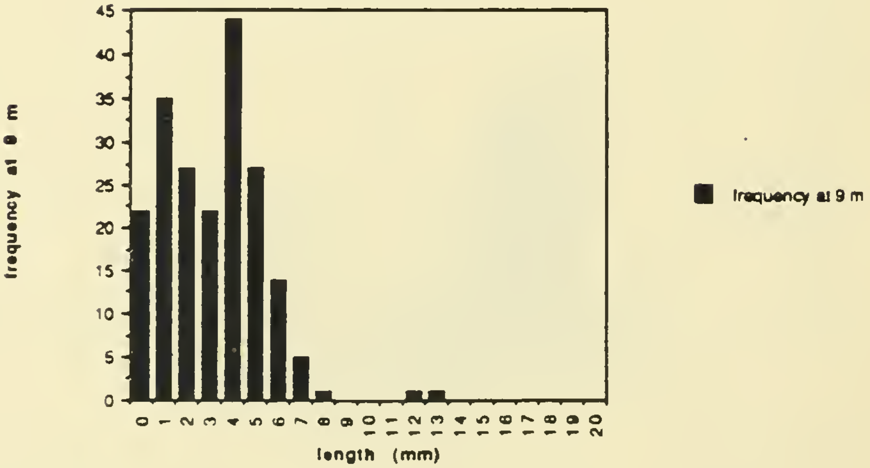
Fig. 4.



Length/frequency distribution of zebra mussels at the 1 m depth in the Pelee Passage (buoy X), Lake Erie, Ont.



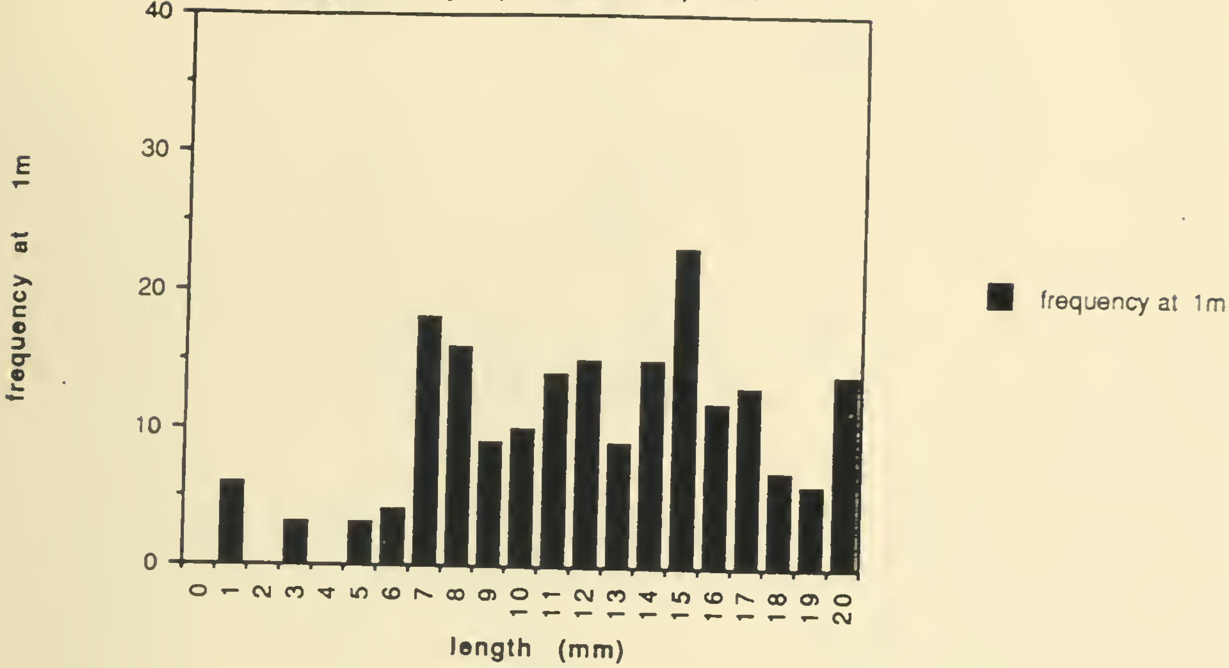
Length/frequency distribution of zebra mussels at the 2 m depth in the Pelee Passage (buoy X), Lake Erie, Ont.



Length/frequency distribution of zebra mussels at the 9 m depth in the Pelee Passage (buoy X), Lake Erie, Ont.

Fig. 5.

Length/frequency distribution of zebra mussels at the 1 m depth in the Pelee Passage (buoy P), Lake Erie, Ont.



Length/frequency distribution of zebra mussels at the 4 m depth in the Pelee Passage (buoy P), Lake Erie, Ont.

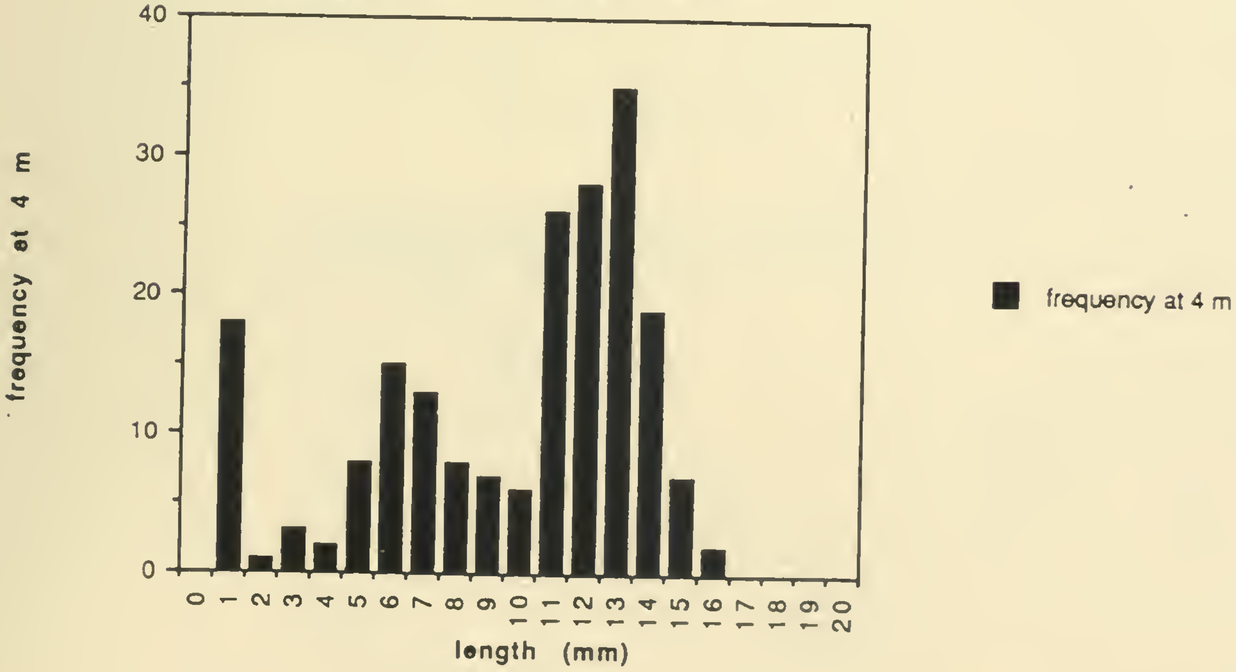
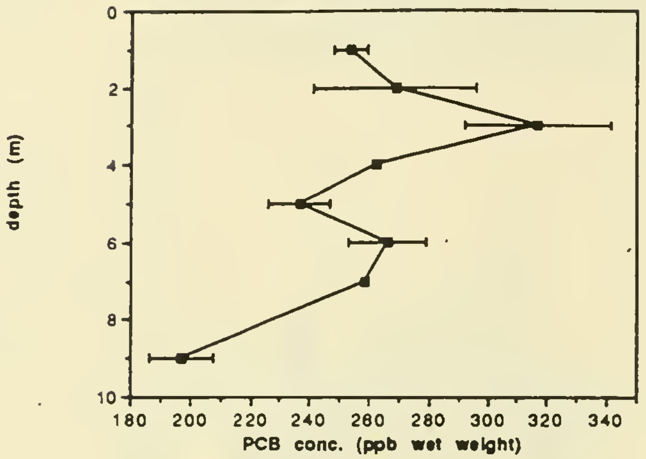
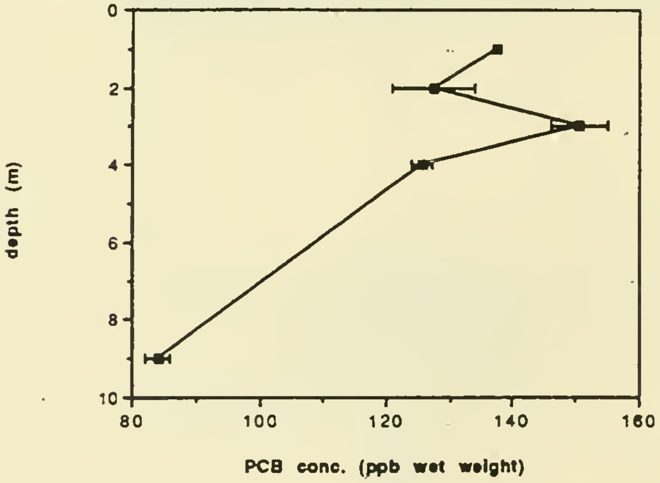


Fig. 6.

PCB concentrations (per unit wet weight) in zebra mussels in relation to depth, Toledo Harbour, Lake Erie, Ohio (± range, n = 2).



PCB concentrations in zebra mussels (per unit wet weight) in the Pelee Passage (buoy P), Lake Erie, Ont. (± range, n = 2).



PCB concentrations in zebra mussels (per unit wet weight) in the Pelee Passage (buoy X), Lake Erie, Ont. (± range, n = 2).

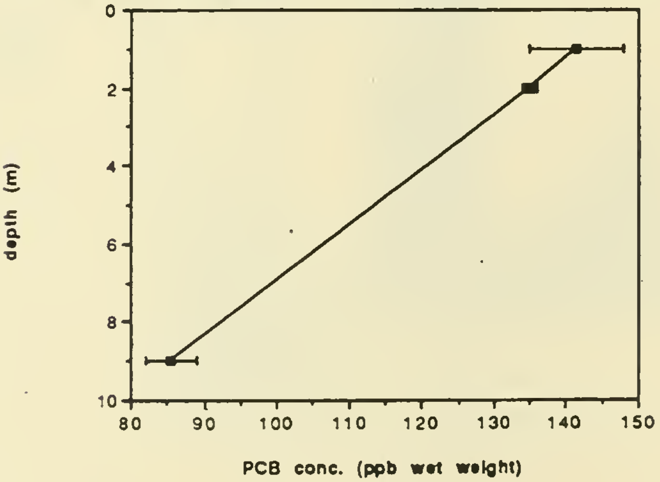
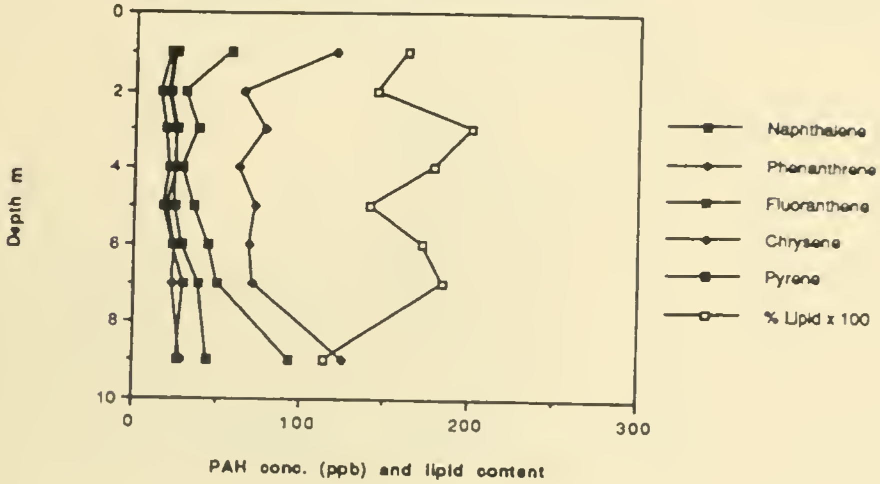
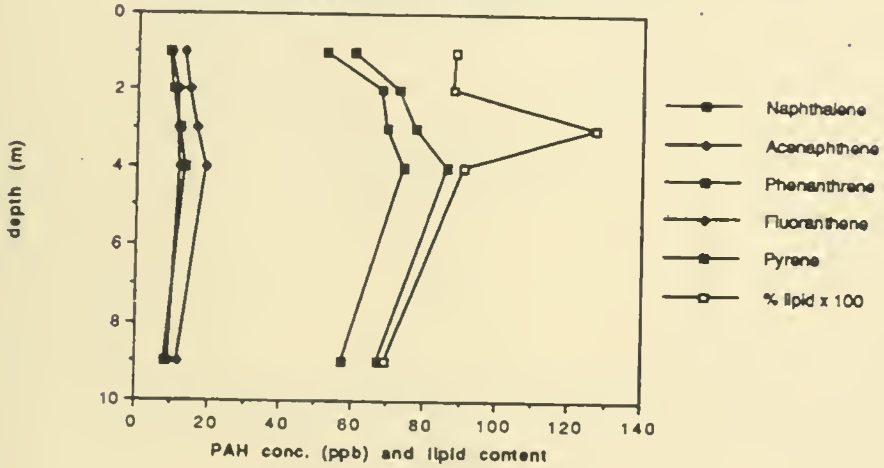


Fig. 7.

PAH concentration and lipid content in zebra mussels in relation to depth in Toledo Harbour, Ohio (n = 2).



PAH concentrations in zebra mussels in relation to depth in the Pelee Passage (buoy P), Lake Erie, Ont. (n = 2).



PAH concentration and lipid content in zebra mussels in relation to depth in the Pelee Passage (buoy X), Lake Erie, Ont. (n = 2).

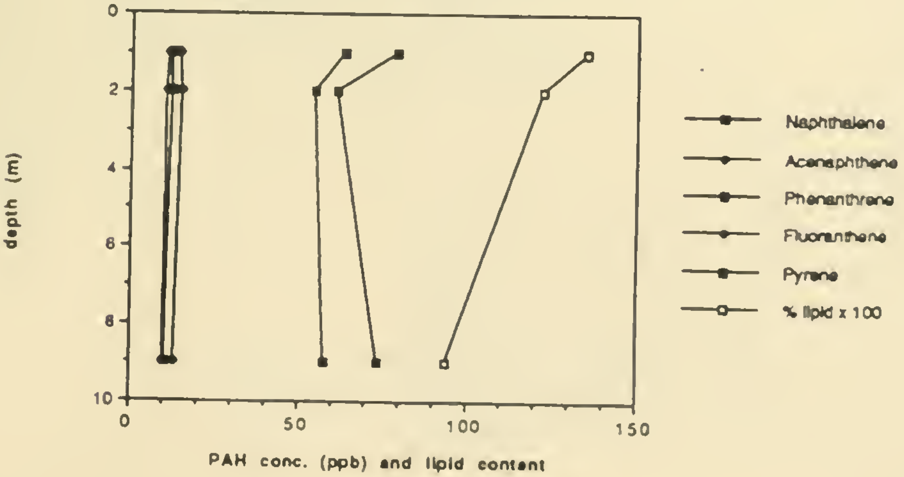
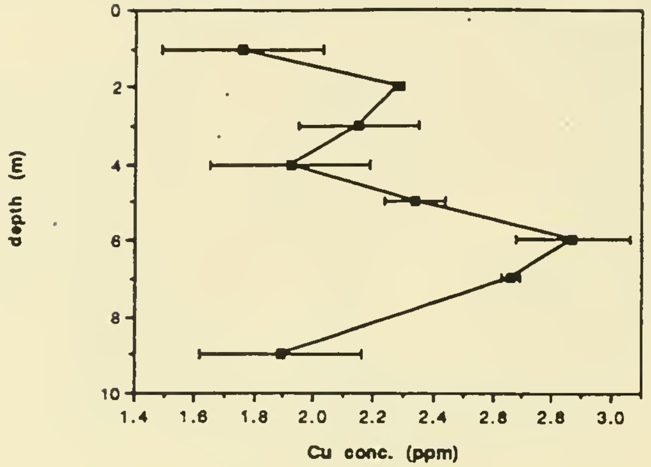
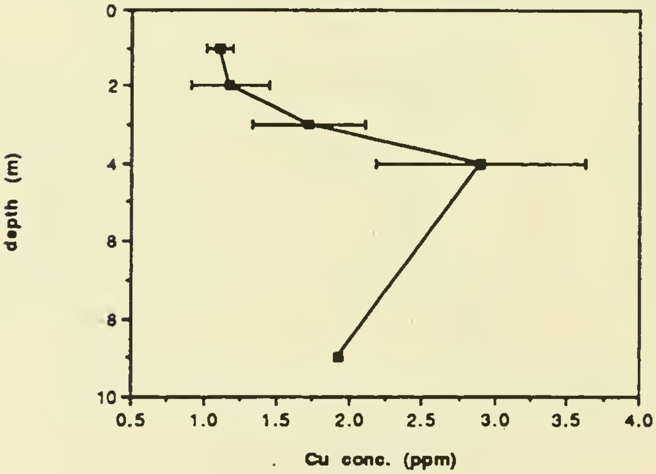


Fig. 8.

Cu concentrations in zebra mussels in relation to depth in Toledo Herbour, Ohio (± range, n = 2).



Cu concentrations in zebra mussels in relation to depth in the Pelee Passage, buoy P, Lake Erie, Ont. (± range, n = 2)



Cu concentrations in zebra mussels in relation to depth in the Pelee Passage, buoy X, Lake Erie, Ont. (± range, n = 2).

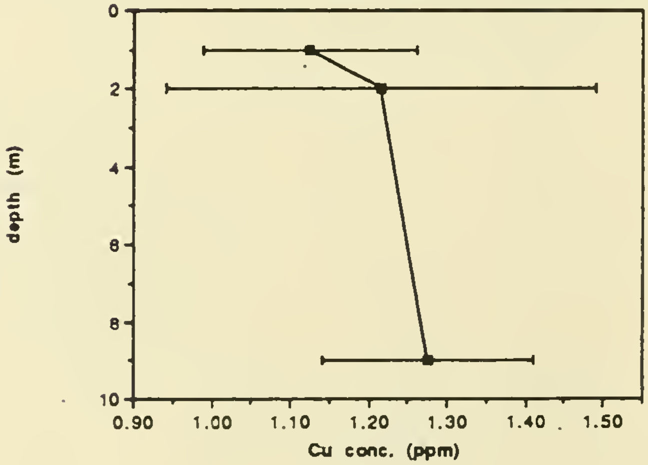
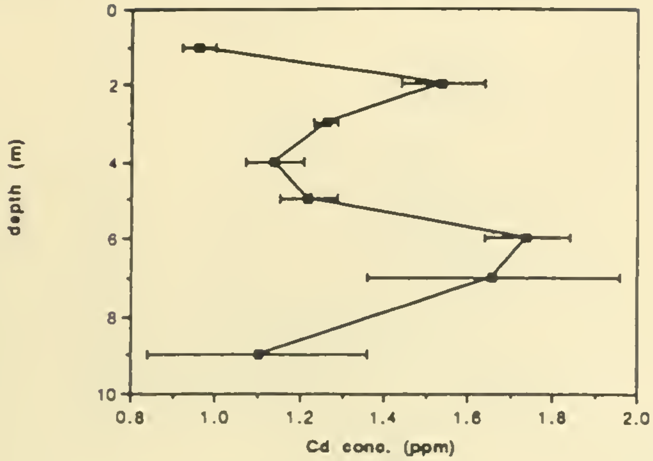
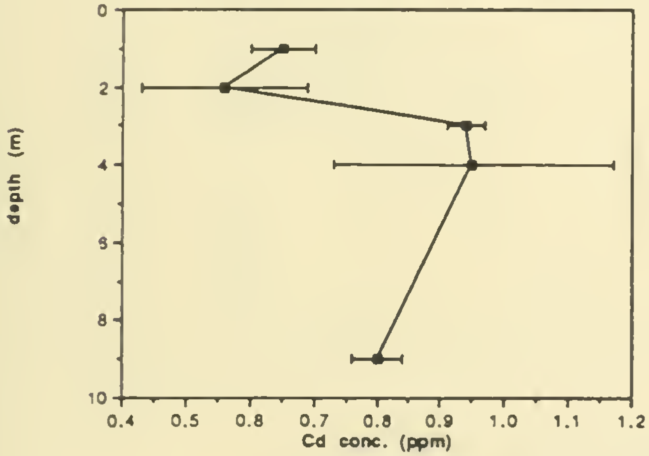


Fig. 9.


Cd concentration in tissues of zebra mussel in relation to depth in Toledo Harbour, Ohio (± range, n = 2).


Cd concentration in tissues of zebra mussels in relation to depth in the Pelee Passage, buoy P, Lake Erie, Ont. (± range, n = 2).

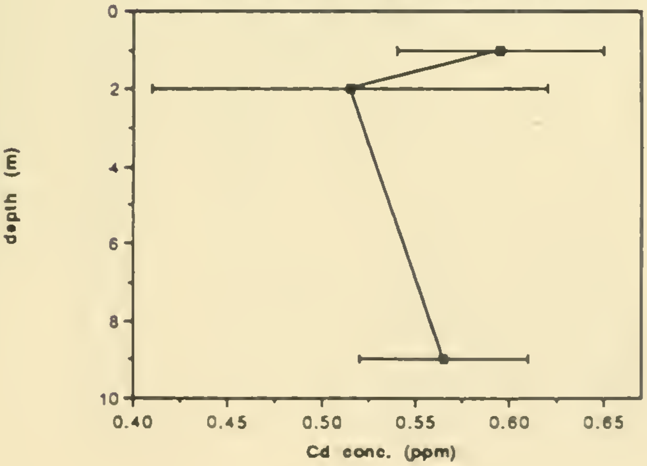
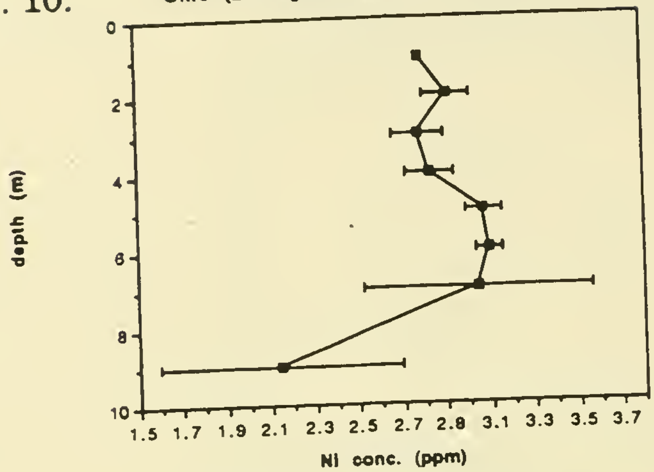
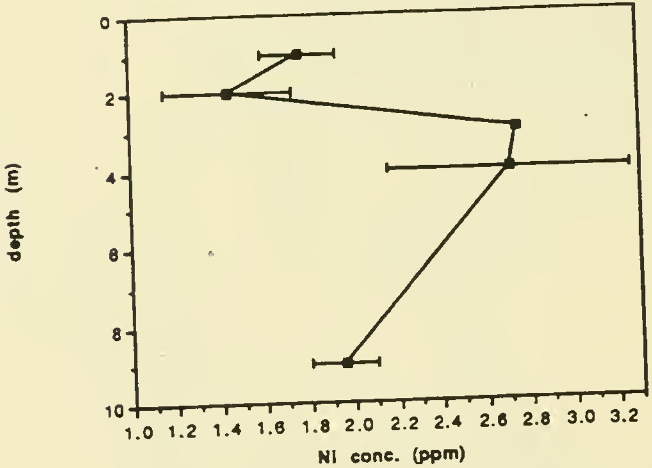

Cd concentration in zebra mussels in relation to depth in the Pelee Passage, buoy X, Lake Erie, Ont. (± range, n = 2).

Fig. 10.

Ni concentrations in zebra mussels in relation to depth in Toledo Harbour, Ohio (± range, n = 2).



Ni concentrations in zebra mussels in relation to depth in the Pelee Passage, Buoy P, Lake Erie, Ont. (± range, n = 2).



Ni concentrations in zebra mussels in relation to depth in the Pelee Passage, Buoy X, Lake Erie, Ont. (± range, n = 2).

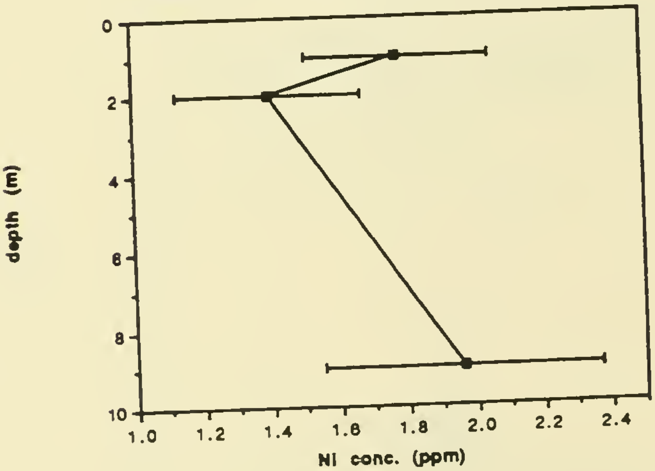
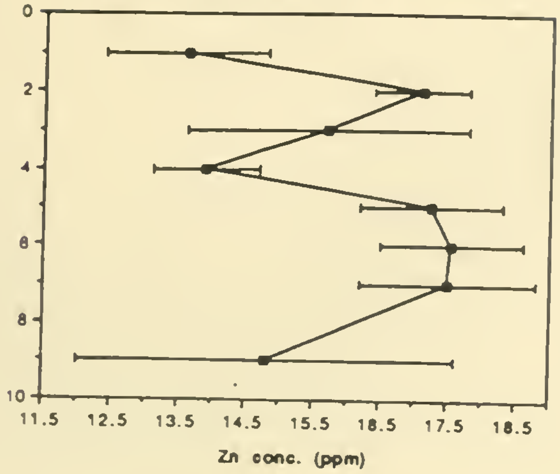
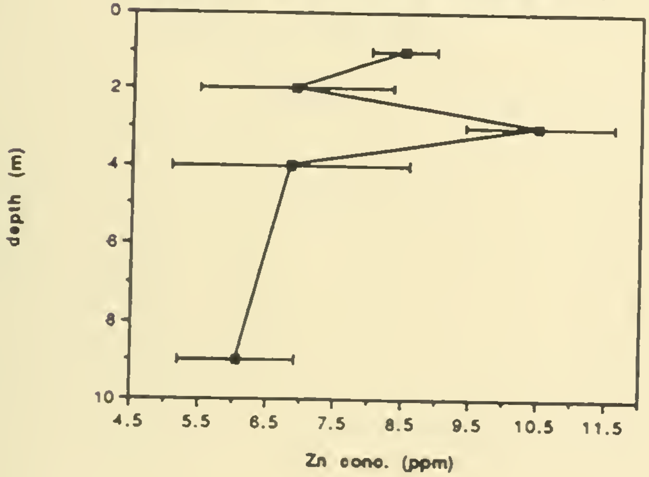


Fig. 11.

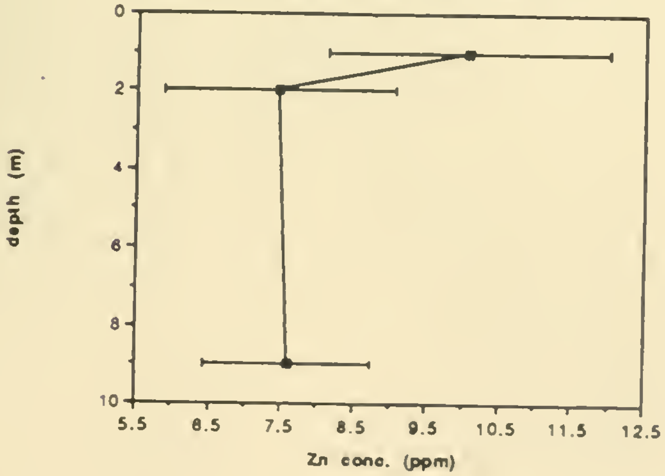
Zn concentrations in zebra mussels in relation to depth in Toledo Harbour, Ohio (± range, n = 2).



Zn concentrations in zebra mussels in relation to depth in the Pelee Passage, Buoy P, Lake Erie, Ont. (± range, n = 2).



Zn concentrations in zebra mussels in relation to depth in the Pelee Passage, buoy X, Lake Erie, Ont. (± range, n = 2).



APPENDIX I

ANALYTICAL METHODOLOGY

1) Organics Analysis

The samples were analyzed for organics according to the methodology being used currently for MOEE fish analyses.

The sample (approx. 5 g fish homogenate or the total zebra mussel sample accurately weighed) is acid digested with concentrated HCL, double extracted with 25% methylene chloride in hexane, and the pooled extracts are neutralized with $NaHCO_3$ and made to 100 ml volume with the solvent mixture. Aliquots are taken for analysis of PCBs/CBs, PAHs and lipid content determination (other aliquots can also be taken for OC pesticide and chlorophenol determination if required). Note that for this batch of 16 samples, an aliquot was taken for metal analysis just after the acid digestion.

a) Analysis for PCBs/CBs.
This is carried out by cleanup/separation on 2% water-deactivated Florisil and analysis by dual capillary GC/EC.

b) PAH Analysis.
This is carried out by GPC cleanup and analysis by capillary GC/MSD.

2) Trace Metal Analysis

Samples were analyzed for trace metals by accurately weighing the mussel tissue (approx. 1g) (as received) into an Erlenmeyer flask. The samples were digested with nitric and hydrochloric acids (5 ml) and 0.2 ml of sulfuric acid. The digested samples were diluted to volume (25 ml) with deionized water and analyzed by inductively coupled plasma emission spectroscopy (ICP).

The 16 samples submitted above for organic analysis were also used for metal analysis. They were treated as follows. The mussel tissue (approx. 5 g) was initially extracted with 50 ml of hydrochloric acid. A 10 ml portion of this extract was accurately measured into Erlenmeyer flask and digested as above and analyzed by ICP.